ENDOCRINOLOGY

VOLUME 36 APRIL, 1945 NUMBER 4

THE OXIDATION-REDUCTION POTENTIAL OF THE THYROID FOLLICLE IN NORMAL AND EXPERIMENTAL CONDITIONS

E. DE ROBERTIS[1] AND J. MOURA GONÇALVES

From the Laboratory of Biophysics, University of Rio de Janeiro, Brazil

THE DISCOVERY that a series of substances such as thiourea and sulfa-compounds inhibit thyroid secretion (Mackenzie, Mackenzie and McCallum, 1941; Kennedy, 1941; Astwood, Sullivan, Bissell and Tyslowitz, 1943) has opened a new field in thyroid physiology and a better approach to the study of the mechanism of synthesis of the thyroid hormone.

In this connection, the study of the oxidation-reduction potential of the thyroid follicle, and especially of its two fundamental constituents, thyroid epithelium and colloid secretion, in the normal state and after the action of inhibitors such as those mentioned above, was particularly interesting. This is so because the level of the oxidation-reduction potential is determined by the activity of the enzymatic systems located in the cells and the colloid, by the O_2 pressure, and by the nature and concentration of the substrates (Stiehler and Flexner, 1938); such a study may also indicate how energy of oxidation can be utilized in the secretory mechanism.

We undertook therefore to investigate the oxidation-reduction potential under various conditions, including activation of the secretory mechanism of the thyroid gland by such agents as thyreotropic hormone of the pituitary gland and low temperature.

It was found that in the normal thyroid follicle there is a wide difference between the oxidation-reduction potential of the cells and

Received for publication January 18, 1945.

[1] Institute of General Anatomy and Embryology, University of Buenos Aires, Argentina.

Copyright, 1945, by the Association for the Study of Internal Secretions.

XUM

the colloid, that this difference disappears in the activated gland, and that under the action of thiourea there is a drop in the level of the oxidation-reduction potential of the thyroid cells.

METHOD

All the experiments were carried out on young adult white rats fed on bread and milk to which halibut oil and yeast were added. For the determination of the oxidation-reduction potential the colorimetric technique was used by two different methods. The microdissection technique enabled us to extract the colloid and to mix it directly with the indicator, or to inject the indicator into the follicle. The other method was the supravital technique described by Friedenwald and Stiehler (1938), which permits determination of the potential of the follicle within the gland tissue.

As activators we used exposure to cold and Antuitrin T (thyreotropic hormone of the pituitary gland) intraperitoneally injected. The action of inhibitors was studied either in vitro or after injection into the animal.

Microdissection technique. Rats were anesthetized with Nembutal and the thyroid gland exposed. The follicles of the isthmus were illuminated and observed under a binocular microscope (×100 diameters). Micropipettes with a bore of 5–10μ at the point were adapted to a microinjector and moved with a Peterfi microdissection apparatus, put in a reversed position above the animal.

Indicators were dissolved in isotonic phosphate buffer of pH 6.98. The concentration was just strong enough to permit clearly seeing the color of the fluid in the micropipette. A small quantity of the indicator (approximately 2×10^{-6} ml) was sucked into the pipette and afterwards the colloid was extracted from the follicle and mixed with the indicator within the pipette.

We adopted a time-limit of 10 minutes for the disappearance of the color because when the E_0' of the indicator was above the level of the oxidation-reduction potential of the colloid, the decolorization was completed in less time.

Injection of the indicator into the follicle was also tried but its diffusion made the observation rather difficult. However some interesting features of the behavior of the thyroid cells could be observed.

Supravital method. Rats were killed by a blow on the head and immediately bled by opening the chest. The thyroid glands were dissected with glass needles and forceps made of bone, because it was found a cause of error to put the gland in contact with metal. The glands, placed in a Ringer solution of pH 7, were divided into small pieces with glass needles, in order to see the follicles clearly under the microscope.

The indicators were dissolved in Ringer (pH 7) at a concentration of M/1000. Thionin was dissolved in Sörensen's phosphate buffer of pH 5.8. A drop of the indicator was put on a slide together with a bit of tissue and observed under the microscope for 5 to 10 minutes. The reduction of the color was checked by restoring it with potassium ferricyanide. As the degrees of reduction were difficult to see, results were considered only as positive (+) or negative (−). In addition to the indicators shown in Table II, indigo tri- and tetrasulphonate were used, but they apparently did not penetrate into the tissue.

RESULTS

1.—*The oxidation-reduction potential of the follicular colloid determined by the microdissection technique.*

a) Normal gland: In Table I the action of follicular colloid upon a series of indicators is recorded. The reduction of the color (+) is complete from thionin (E_0'+0.100 volts at pH 6) to cresyl violet (−0.167 volts). On the contrary, phenosafranine (−0.252 volts) and neutral red (−0.330) are not reduced at all. The level of the oxidation reduction potential must be somewhere between −0.167 and −0.252 volts, probably near −0.200 volts.

By injection of the indicator into the follicle it was observed that phenosafranine is not reduced by the colloid nor by the cells. On the contrary, methylene blue (0.011 v.), indigo-tetrasulphonate (−0.046 v.), Nile blue (−0.122) and cresyl violet (−0.167) when injected are reduced by the colloid. Thyroid cells do not reduce methylene blue, Nile blue, and cresyl violet, which shows that their potential is above that of the colloid.

TABLE I. OXIDATION-REDUCTION POTENTIAL OF THE COLLOID DETERMINED BY MICRODISSECTION

Indicators	E_0' at pH 7	Normal rats	Activated rats	Action of KCN in activated rats	Action of thiourea in activated rats
2-6 dichlorophenol-indophenol	+0.217		+		
Thionin (Lauth's violet)	+0.100[1]	+	+		
Cresyl blue	+0.047	+	−		
Methylene blue	+0.011	+	−	+	+
Indigo Tetrasulfonate	−0.046	+	−	+	+
Indigo Trisulfonate	−0.081	+			
Nile blue	−0.122	+	−	+	+
Cresyl violet	−0.167	+	−	+	+
Phenosafranine	−0.252	−	−	−	−
Neutral red	−0.330	−	−	−	−

[1] E_0' at pH 6.0.

b) Glands activated with thyreotropic hormone: The injection of 5 units of Antuitrin T produces a rise in the oxidation-reduction potential of the colloid from approximately −0.200 volts to more than +0.050 volts (Table I). Only thionin at pH 6(+0.217) and 2-6 dibromophenol-indophenol (+0.217) and 2-6 dichlorophenol-indophenol (+0.217) are reduced by the colloid. In several cases the normal potential was determined and afterwards the rat was injected with the hormone and the rise of potential checked.

c) Activation by the action of cold and recovery to the normal state: Activation of the thyroid gland occurred in a group of rats left during two days in the open during windy and cold weather (temperature 8° C.) Thyroid follicles showed an intense congestion, similar to that produced with thyreotropic hormone and the potential

rose to the same level (determined with thionin, methylene blue and indigo-tetrasulphonate). Recovery from activation and return to the normal potential level was obtained after keeping the rats 4 days at 28° C.

d) Action of KCN on the oxidation-reduction potential of the activated gland: Several experiments were done with a solution of KCN (M/100) in a buffer of pH 7 to which the indicators were added. The rats were activated with thyreotropic hormone and by cold, and the high level of the potential was first determined. As shown in Table I, KCN lowered the potential to the level found in normal glands (below −0.167 volts).

e) Action of thiourea on the oxidation-reduction potential of the activated gland: The action of thiourea was studied in vitro and in vivo. In the first case indicators were dissolved in a solution of thiourea (M/200), and in the second 8 to 10 mgs. of thiourea were

TABLE II. OXIDATION-REDUCTION POTENTIAL OF THYROID AND COLLOID DETERMINED BY SUPRAVITAL TECHNIQUE

Indicators	E_0' at pH 7	Normal rats		Activated rats		Action of KCN		Action of thiourea		Action of sulfamide	
		cells	colloid	cells	colloid	cells	colloid	cells	colloid	cells	colloid
Thionin (Lauth's violet)	+0.110[1]	+	+	+	+	+	+	+	+	+	+
Cresyl blue	+0.047	−	+	−	−	+	+	+	+	−	+
Methylene blue	+0.011	−	+	−	−	+	+	+	+	−	+
Nile blue	−0.122	−	+	−	−	+	+	+	+	−	+
Cresyl violet	−0.167	−	+	−	−	+	+	+	+	−	+
Phenosafranine	−0.252	−	−	−	−	−	−	−	−	−	−
Neutral red	−0.330	−	−	−	−	−	−	−	−	−	−

[1] E_0' at pH 5.8.

injected. In both cases a drop of the oxidation-reduction potential to the level of the normal glands was observed.

2.—*The oxidation-reduction potential of the thyroid cells and the colloid determined by the supravital method.*

a) Normal glands: The level of the oxidation-reduction potential of the cells lies somewhere between +0.047 volts and +0.110 volts (Table II) while in the colloid it is between −0.167 and −0.252 volts. The potential of the stroma could not be determined accurately but it certainly is much lower than in the thyroid cells, being probably near that of the colloid.

b) Activated glands: In rats injected with thyreotropic hormones the potential of the thyroid cells apparently does not change, but in the colloid it is greatly increased and reaches the same level as in the cells. This is in accordance with the results obtained by microdissection.

c) Action of KCN on normal glands: The action of KCN was studied by putting the glands in a M/100 solution of KCN in Ringer and afterwards in the indicator. As may be seen from Table II, KCN lowers the potential of the cell to less than −0.167 volts.

d) Thiourea, injected into the animal and afterwards applied supravitally in the same concentration as KCN, also lowers the oxidation-reduction potential (Table II).

e) Action of sulfanilamide: Rats were injected with 35 mg. of p-aminophenylsulfamide. Two hours later the thyroid glands were removed and dissected in M/200 solution of the sulfa compound. In several cases the indicators were also dissolved in the sulfa solution. It was found that the oxidation-reduction potential was apparently not affected by the action of sulfanilamide (Table II).

DISCUSSION

In 1941 one of us (De Robertis, 1941), using a microdissection technique, determined the pH of the follicular colloid in normal and activated glands. No significant changes with activation were observed. Contrary to this finding, the oxidation-reduction potential of the colloid is strongly modified by the activation: normally -0.200 volts, it rises to $+0.050$ volts, i.e. to the E_0' of the thyroid cell.

This change of 0.250 volts in the colloid of an activated gland is probably due to the penetration into the follicle of an enzymatic system which raises the level of the oxidation-reduction potential. This hypothesis is supported by the fact that the action of KCN on the colloid of activated glands lowers the oxidation-reduction potential to the normal level. In another paper several experiments will be described in which an enzymatic system of the oxidative type was found present in the colloid of activated glands but absent in the normal gland.

One of us has described (De Robertis, 1942), from the histological point of view, the penetration into the colloid of parts of the cytoplasm of the thyroid cells, when the gland is activated with thyreotropic hormone. It is interesting to quote in this connection a previous observation made by Okkels (1934) of the penetration of "oxidasic granules" from the cells into the colloid under the action of thyreotropic hormone.

The fact that thiourea and sulfa compounds produce hypertrophy of the thyroid gland with inhibition of the thyroid secretion is now well established. Mackenzie, Mackenzie and McCallum (1941); Kennedy (1941); Astwood, Sullivan, Bissel and Tyslowitz (1943); Franklin and Chaikoff (1944); Keston, Goldsmith, Gordon and Charipper (1944); Couceiro (1944). Franklin and Chaikoff (1944) and Keston and his collaborators (1944) demonstrated that sulfanilamides and thiourea inhibit the conversion of inorganic I into diodotyrosine and thyroxine. Martin (1943), analyzing the action of numerous chemicals which exhibit depressive action upon the thyroid gland, reaches the conclusion that this activity is due to a general inhibition of tissue metabolism. Astwood (1943), studying the chemical nature of thyroid inhibitors, suggests as a working hypothesis that anilin derivatives, such

as sulfanilamides, act by a competitive effect on the enzymatic system, responsible for the conversion of diodotyrosine into thyroxine and that thioureas probably act as specific inhibitors of these systems.

As the aerobic oxidation-reduction potential of the cell depends upon the activity of the different enzymatic systems and substrates which intervene in cellular metabolism, it is interesting to note that thiourea acts on the thyroid gland by producing a marked drop of the oxidation-reduction potential of the thyroid cell. It seems interesting also that another inhibitor of the synthesis of the thyroid hormone, sulfanilamide, does not act by changing the oxidation-reduction potential level of the thyroid cell.

CONCLUSIONS

The aerobic oxidation-reduction potential of the colloid and of the thyroid cells was determined, using indicators in microdissection experiments and supravitally. In the thyroid gland of the normal white rat, the oxidation-reduction potential of the thyroid cells is above +0.050 volts, and in the colloid about −0.200 volts. In glands activated by cold or by injection of thyreotropic hormone of the pituitary gland the oxidation-reduction potential of the colloid rises to the same level as in the cells (+0.050 volts). The oxidation-reduction potential of the thyroid cells and of the colloid of activated glands is lowered under the action of KCN to −0.200 volts. Thiourea acts in vitro and in vivo, lowering the potential of the thyroid cells and of the colloid of activated glands to −0.200 volts. Para-aminophenylsulfamide injected into the animal does not change the oxidation-reduction potential of the thyroid cells.

REFERENCES

ASTWOOD, E. B.: *J. Pharmacol. & Exper. Therap.* 78: 79. 1943.
ASTWOOD, E. B., J. SULLIVAN, A. BISSEL AND R. TYSLOWITZ: *Endocrinolgy* 32: 210. 1943.
COUCEIRO, A.: *Rev. brasil. de biol.* 4: 173. 1944.
DE ROBERTIS, E.: *Anat. Rec.* 80: 219. 1941.
DE ROBERTIS, E.: *Anat. Rec.* 84: 125. 1942.
FRANKLIN, A. L., AND I. L. CHAIKOFF: *J. Biol. Chem.* 152: 295. 1944.
FRIEDENWALD, J. S., AND R. D. STIEHLER: *Arch. Ophth.* 20: 761. 1938.
KENNEDY, D.: *Bril. J. Exper. Path.* 22: 241. 1941.
KESTON, A. S., E. D. GOLDSMITH, A. S. GORDON, AND H. A. CHARIPPER: *J. Biol. Chem.* 152: 241. 1944.
MACKENZIE, C. G., AND J. B. MACKENZIE: *Endocrinology* 32: 185. 1943.
MACKENZIE, J. B., C. G. MACKENZIE AND E. V. MCCALLUM: *Science* 94: 518. 1941.
MARTIN, G. J.: *Arch. Biochem.* 3: 61. 1943.
OKKELS, A.: *Compt. rend. Soc. de Biol.* 116: 251. 1934.
STIEHLER, R. D., AND L. B. FLEXNER: *J. Biol. Chem.* 126: 603. 1938.

A STUDY OF THE EFFECT OF PROLACTIN ON BROODINESS AND ON COCK TESTES

A. V. NALBANDOV
With Assistance of PVT. M. HOCHHAUSER AND MARIE DUGAS
University of Illinois[1]

IN AN EARLIER STUDY an attempt was made to determine the number of genes for broodiness carried by cocks by means of hormone treatment rather than breeding tests (Nalbandov and Card 1945). It was found in that study that sufficient amounts of prolactin will cause males, regardless of their genotypes, to show most of the broody responses shown by broody hens. The amount of prolactin required to bring about this response in males was roughly inversely proportional to the number of genes for broodiness assumed to be carried by them. Regardless of the dosage of prolactin injected none of the males could be induced to incubate eggs, but they will assume complete charge of a group of day old chicks. Tasks usually identified with the broody hen would then be executed by prolactin-treated males who would lead chicks to feed and water, protect and shelter them under body and wing, attack and drive away aggressors and utter a variety of vocal sounds, including clucking, normally not heard from males, but understood and obeyed by the chicks. Uninjected males, when confined with chicks, either kill them or allow them to die of starvation and exposure, because of active antagonism or lack of interest. In view of these findings the question arose how prolactin was capable of invoking these psychological responses.

The role of "hormones in the development of unlearned or instinctive behavior" has been reviewed by Riddle (1942) who accepts Lashley's (1938) summary that "the effective hormones act upon the central nervous system to increase the excitability of the sensorimotor mechanism specifically involved in the instinctive activity."

A series of observations made during preliminary studies by the present author and mentioned in the introduction made it seem desirable to reexamine the conclusions reached by other workers.

In our experiments full broody response was never observed to develop on an "all or none" basis. It was attained gradually and was lost gradually. Thus males, when given five newly-hatched chicks on each of the five days, would react more or less as follows:

1st injection—killed 5 chicks
2nd injection—killed 5 chicks

Received for publication January 20, 1945.
[1] Support of the University of Illinois Research Board is gratefully acknowledged.

3rd injection—killed 3 chicks adopted 2
4th injection—killed 1 chick adopted 4
5th injection—killed 0 chicks adopted 5

Depending on genotype and individuality the total amount of prolactin necessary would vary and also the numbers of chicks neglected or killed would depart from the above example but invariably the broody response would appear gradually and be lost gradually after injections were stopped. Prolactin treatment was accompanied by certain other features which made it seem probable that complete broodiness could not be achieved until the androgen had reached a level lower than that in the untreated normal male. That there was an almost immediate reduction in the amount of androgen circulating activity was seen from the fact that after the first prolactin injection males ceased crowing. After the second or third injections they refused to mate when given the opportunity and quite definitely assumed an inferior status in the hierarchy if kept with normal males, whereas they may have been socially superior before the treatment. Chronologically later, decrease in androgen level was witnessed by a decrease in comb and wattle size. These facts made it seem likely that prolactin acted primarily either by supressing pituitary activity, thus depriving the testes of gonadotrophins and reducing their androgenic activity, or it acted by preventing the androgen from exerting its expected endocrine action. It has been found by Riddle and Bates (1933), Bates, Lahr, and Riddle (1935) and Bates, Riddle and Lahr (1937) that the antigonadal action of prolactin consists in preventing the pituitary from elaborating FSH, thus depriving the gonads of hormonal support and causing far-reaching ovarian and testicular atrophy suggestive of hypophysectomy. That it was an inhibitory effect on the pituitary and not on the testes proper was shown by the fact that small dosages of FSH given concurrently with the lactogenic hormone would prevent "prolactin damage."

EXPERIMENTAL

The animals used in these experiments were S. C. White Leghorn males which had reached sexual maturity. The ages of the cocks varied from six to ten months but comparisons from which conclusions were drawn were made only between birds of the same ages.

The prolactin preparations used in these experiments were kindly made available by Drs. R. K. Meyer and H. W. McShan of the University of Wisconsin. LH preparations were furnished by the University of Wisconsin and by Dr. Schooley of the Difco Company. The prolactin preparation (Lac R 200 W) when tested by the crop-gland method on pigeons was found to be lactogenically potent producing crop glands weighing three to eight times more than those of controls. When tested on immature female rats it was found to produce average ovarian weights of 12.0 mgs. with a "+" stimulation of the uteri and closed vaginae. Thus Lac R 200 W is either free from or low in FSH.

The technique of prolactin injection was the same in all cases. The powder was suspended in distilled water with NaOH added to increase solubility. Each bird was given two intramuscular injections daily (A.M. and P.M.) for periods of twelve or thirty days, autopsy being performed on the 13th and 31st days respectively. Regardless of duration of the injection period the daily dosage was the same for all birds. Each cock was given daily an amount equivalent to 6.0 grams of dried pituitary powder or actually 214.8 mgs. of Lac R 200 W in twelve days. These rather high dosages were chosen because preliminary trials showed that they will bring about the most complete broody response from all males regardless of their genotypes.

At the time of the first and last injections or at autopsy, body weight, comb area (length×height) and testes weights were determined. In order to study the effect of the prolactin treatment upon the diameter of the seminiferous tubules and the proportions of these to the intertubular tissue the following procedure for both treated and control testes was adopted. A mounted section of a testicle fixed in FAA, cut at 8μ and stained in Mallory's triple stain was placed under the microscope and a field selected at random. By means of a projection attachment the image was thrown upon a piece of paper measuring 5×8 inches and lying on the table in front of the microscope. A magnification was chosen which would permit about twenty tubule images to appear on the paper. The tubule outlines (easily visible in semi-darkness) were carefully traced with a hard pencil. To determine the tubule diameter fifteen of these outlined tubules were measured with a planimeter. The figures thus obtained are, of course only relative; but since the actual diameter is not an issue in this study this method being decidedly simple and accurate is preferable to the frequently used method of tracing and cutting out tissue outlines with subsequent weighing of the cuttings. If actual measurements are wanted these can easily be obtained by making a few calculations.

The same method was applied in determining the relative proportions of tubule area to interstitial area. Here a circle with a radius of $2\frac{1}{4}$ inches was drawn over the tubule images used in the above measurements. The total area occupied by whole tubules or portions of tubules and the total area occupied by intertubular tissue falling within the circle were measured with a planimeter and their proportions to the circle area calculated.

RESULTS

The data obtained confirm the known inhibitory effect of prolactin upon the gonads. In this experiment testis size was decreased to 56 per cent of normal following the twelve-day injection period and to 26 per cent of normal after the thirty-day period (Table I). Decrease in testis size led to decreased androgenic activity which in turn resulted in decreased comb size, cessation of crowing, social inferiority and lack of sex drive.

In addition to these effects prolactin treatment also caused a significant decrease in tubule diameter. This effect is presumably due to the shutting off of the hypophyseal FSH. The proportions of tubular and intertubular areas do not differ following the short time injection but there is a decrease in tubule area following the treatment lasting

for thirty days (Table I). In other words, the proportional amount of intertubular tissue is double that of the control testes. This, however, does not necessarily mean that there is an actual increase in the glandular elements of the interstitium. The possible significance of this change will be treated below.

The findings of Bates *et al.* (1933, 1935, 1937) that prolactin exerts its inhibitory effect indirectly through the pituitary have been verified. If even a small amount of FSH is injected along with prolactin no decrease in testis size or tubule diameter and no changes in the pro-

TABLE I. EFFECT OF PROLACTIN ON TESTIS WEIGHT, TESTIS COMPONENTS AND COMB AREA OF ADULT MALES

Items measured	Injected prolactin	Control	Injected prolactin	Control
No. of days injected or used as controls	12	12	30	30
No. of animals used	14	14	5	5
Testes weight (grams)	5.73 ± 4.76[1]	10.12 ± 2.41[1]	3.2 ± 1.3[1]	12.15 ± 2.59[1]
Comb area (Length × height) (cm2)	74.8 ± 14.1	83.6 ± 12.5	52.3 ± 6.35[1]	95.7 ± 12.6[1]
Tubule diameter	393.4 ± 180.2[1]	560.9 ± 174.0[1]	192.5 ± 53.8[1]	600.5 ± 183.0[1]
Per cent of testis area tubules	90.3 ± 3.1	89.2 ± 4.6	75.8 ± 2.99	87.9 ± 4.6
Per cent of testis area intertubular tissue	9.7	10.8	24.2	12.1
No. of tubules in measured area	34.9 ± 9.4	23.1 ± 6.8	45.5 ± 9.5	22.9 ± 7.5

[1] Differences significant statistically $p = .01$.

portionate relationships in the testes elements were observed. No adverse effects upon either psychological or physiological maleness resulted.

A corollary of the above experiment was also performed in which a small amount (0.1 mg) of an androgen (methyltestosterone) was injected once daily along with the prolactin. This treatment had the same effect as did the FSH; it prevented the males from showing any symptoms of broodiness which would normally be observed if the androgen treatment had been omitted. This indicates that prolactin cannot start the chain of events leading to complete broodiness as long as androgen is present.

The above experiments indicate that broodiness in males is contingent on the absence of androgen. Since, according to the prevalent assumption, androgens are secreted by the Leydig cells of the interstitium, and since these in turn are supposed to be supported by LH it is logical to assume that it should be possible to prevent reduction of androgenic activity of the testes by the injection of LH along with prolactin. (The dosages of LH injected were sufficiently large to cause from one to three ovulations in FSH pretreated hens.) In spite of injections of large dosages of LH from two different sources (Difco and University of Wisconsin) we were unable to prevent broodiness or to

diminish its manifestations in any of the five males used in the experiments involving LH and prolactin. LH injected by itself is incapable of inducing broodiness.

DISCUSSION

The experiments presented above throw light upon two unrelated questions. One is the source of androgens in the testes, the other the

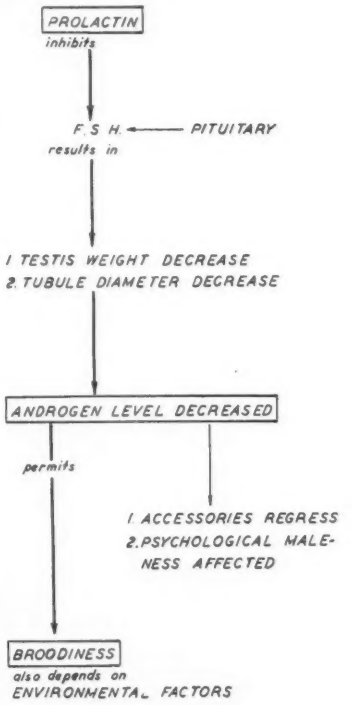

FIG. 1. Events pictured may be thought to occur in the following order.
1. Environmental factors may cause release of prolactin (neural mechanism?) which
2. is capable of preventing FSH from acting thus causing a
3. decrease in androgen secretion (see text) which in turn, in combination with certain environmental factors causes
4. broodiness.
(For details see text).

endocrine basis of a type of behavior, namely, broodiness. From our experiments it appears that the lactogenic hormone in itself does not bring about broodiness. The chain of events as visualized by us is presented in Figure 1. It appears reasonable to assume that broodiness is the result of two factors: 1. the absence of androgens (or broadly speaking absence of sex hormones) and 2. a certain group of

environmental stimuli which are essential for complete broody response. Goodale (1916) has shown that capons, when properly conditioned, will show complete broody response and will perform duties similar to those of a broody hen without the injection of any hormones. Burrows and Byerly (1938) have shown that hens and pullets when exposed to warm temperature (90°F.), complete darkness and the presence of chicks will become broody within eighteen hours to twelve days after the above mentioned change in environmental conditions. Our evidence together with the results of Goodale and Burrows would make the following theory plausible: environmental factors such as light-intensity, temperature, and psychological factors such as accumulation of eggs in the nest and/or the presence of chicks necessary to bring about a change in the secretory activity of the pituitary (Fig. 1). On this basis two types of broodiness are theoretically possible. One type is that in which broodiness is the result of inactivity of the gonad (due to lack of hypophyseal support) plus proper environmental stimuli. The other type of broodiness may be postulated to be in its end result the same as the first type except that here the chain of events is started off by the presence of lactogenic hormone rather than the primary absence of FSH.

In this case one can think of the psychological or neurophysiological stimuli (eggs in nest or chicks) as causing the release of prolactin from the pituitary gland, which in turn would cause the selective shutting off of FSH. From then on events might occur as visualized in the diagram.

Riddle, Lahr and Bates (1942) used a variety of hormones in studies on the maternal instinct in rats. They found that many pituitary hormones (except pregnant mare serum), several steroidal hormones, phenol and thyroxine are all capable of inducing the instinct in normal, thyroidectomized, hypophysectomized and castrated males and females. From their studies it appears that it is much easier to initiate the maternal instinct in rats than to cause broodiness in chickens. In chickens we have definitely found that whole anterior pituitary, LH, FSH, testosterone, estrogen and progesterone when given by themselves are completely incapable of causing broodiness. All of the above named substances (and many others) appear to be effective in the rat. Riddle and Lahr (1944) working with ring doves reported that progesterone, desoxycorticosterone and testosterone propionate induced both males and females to brood eggs and to rear young. These authors indicate that they consider broodiness in the dove to be the result of the direct action of prolactin. In view of the fact that no amount of prolactin will cause chicken males to "brood eggs" and that none of the other hormones (especially not testosterone) tested on chickens will cause broodiness we are led to believe that broodiness in chickens is a much more complicated and a more highly specialized response than it is in the dove. While Riddle and

Lahr explain the effectiveness of steroidal hormones in producing broodiness in the dove on the basis that these hormones cause the release of prolactin from the pituitary, our results indicate that broodiness in the chicken is the result of the antigonad action of prolactin and is thus due in a large extent to the absence of hormones rather than to their presence. Supporting this theory are the facts that the capon becomes broody solely after proper environmental conditioning and the hen becomes broody only after ovarian function has ceased.

Results obtained in our experimental work may be summarized as follows:

1. If FSH is injected along with prolactin the effect of prolactin is nullified.
2. If androgen is injected along with prolactin the effect of prolactin is nullified.
3. If LH is injected along with prolactin effect of prolactin on psychological and physiological maleness is unimpaired.

From these data it appears either that prolactin is capable of selectively shutting off FSH, or that prolactin may prevent FSH from exerting its normal action.

The fact that the major effect of lack of FSH is seen in a decrease of tubule diameter and eventually a decrease of the proportion of testes tubules to interstitial tissue (Table I) has suggested the possibility that the bulk if not all of the androgenic activity is due to the tubules rather than to the cells of Leydig. While this question is being investigated more exhaustively in another experiment and with different material (hypophysectomized males) it is of interest to point out some implications arising from the experiment under discussion. If simultaneous injection of prolactin and androgen or prolactin and FSH nullifies the action of prolactin, the assumption is valid that FSH stimulates the androgen secreting tissue to supply its own sex hormone. To test this assumption further it is found that LH, which is traditionally assumed to be able to stimulate interstitial tissue, is not capable of preventing the effects of prolactin which are easily overcome with even a small amount of androgen. Thus the conclusion that must be reached from these experiments is that LH, at least in the chicken, does not maintain the cells of Leydig, or that the cells of Leydig have only very limited if any androgenic (see also Breneman (1936), activity. Evidence supporting these conclusions is being accumulated at present from work with completely hypophysectomized males.

SUMMARY AND CONCLUSIONS

Injection of prolactin into normal, sexually mature cocks has verified the earlier findings that this hormone inhibits the gonads by selectively shutting off hypophyseal FSH.

Reduction of testes weight is accompanied by decrease in androgenic activity which in turn is reflected in changes in psychological (absence of crowing, treading, etc.) and physiological (comb area) maleness. Histologically it is found that there is a reduction in tubule diameter to as much as 32 per cent of normal and actually an increase in the proportion of intertubular tissue.

It is postulated that broodiness in males is not brought about primarily by the presence of lactogen but is due to the absence of male sex hormone and the presence of certain environmental stimuli. These results are being checked on hens.

Psychological and physiological action of lactogenic hormone is nullified if prolactin is injected together with androgen or FSH but remains unimpaired if injected with LH. From these data the assumption is made that in the chicken LH does not maintain cells of Leydig causing them to secrete androgen or alternatively that androgen is being secreted by a component of the seminiferous tubules rather than by the Leydig cells. These findings are being further studied on hypophysectomized males.

REFERENCES

BATES, R. W., E. L. LAHR AND O. RIDDLE: *Am. J. Physiol.* 111: 361. 1935.
BATES, R. W., O. RIDDLE AND E. L. LAHR: *Am. J. Physiol.* 119: 610. 1937.
BRENEMAN, W. R.: *Anat. Rec.* 64: 211. 1936.
BURROWS, W. H., AND T. C. BYERLY: *Poul. Sci.* 17: 324. 1938.
GOODALE, H. D.. *J. Anim. Behavior* 6: 319. 1916.
LASHLEY, K. S., *Psych. Rev.* 45: 445. 1938.
NALBANDOV, A. V., AND L. E. CARD. *J. Hered.* in press. 1945.
RIDDLE, O., AND R. W. BATES. *Endocrinology* 17: 689. 1933.
RIDDLE, O., E. L. LAHR AND R. W. BATES. *Am. J. Physiol.* 137: 299. 1942.
RIDDLE, O.. *Symp. on Quant. Biol.* 10: 7. 1942.
RIDDLE, O., AND E. L. LAHR. *Endocrinology* 35: 255. 1944.

THE METABOLISM OF RATS AFTER THYROIDECTOMY OR DURING THIOURACIL TREATMENT, AND THE EFFECT OF THYROID FEEDING

ARTHUR E. MEYER AND G. VIRGINIA RANSOM
From the Research Laboratories, The Maltine Company
BROOKLYN, NEW YORK

As REPORTED by Meyer and Wertz (1939), the thyroidectomized rat is an excellent animal for studying the metabolic effect of thyroid material. Since it has been shown recently by a large number of investigators that thiouracil inhibits the formation of the metabolic hormone in the gland, thus producing a reduction in metabolic rate similar to thyroidectomy, it appeared feasible to use thiouracil-treated in place of thyroidectomized animals in bioassay. In our experience involving several thousands of rats, we have found that a percentage of the rats (we estimate 5%) do not show the expected decline in metabolic rate after thyroidectomy; there may be no noticeable decrease in metabolic rate, or the decrease may be of moderate degree with a tendency to return to the pre-operative level.

Post mortem examination of these rats usually showed that the thyroidectomy had been complete, but occasionally accessory thyroid tissue could be found. Such animals are useless for assay purpose and had to be discarded. These exceptions in metabolic behavior caused by accessory thyroid tissue should not be found in animals treated with thiouracil, which influences any thyroid tissue regardless of its location in the body.

METHOD

Male rats, approximately 80 days of age, were standardized for metabolic and heart rates and then either thyroidectomized or placed on thiouracil. The drug was mixed at a rate of 0.05 per cent with the diet, consisting of 3 parts wheat and 1 part dried whole milk. In addition, the drinking water was saturated with thiouracil. This latter precaution was taken to assure a continual intake of the medication because it is not known how long it takes for a thyroid to become active again when the treatment with thiouracil is interrupted, and because the rats were starved for 18–20 hours preceding the metabolic test. The metabolic rate and heart rate were determined weekly after thyroidectomy or after the beginning of thiouracil treatment until the athyroid level had definitely been reached. The metabolism was determined in a closed circuit Benedict apparatus as described by Meyer and Wertz (1939). The heart rate was recorded with a Garceau electroencephalograph. On this occasion we wish to stress the necessity of having the animal abso-

Received for publication February 5, 1945.

lutely at ease when the heart beat is taken. We obtained satisfactory quiescence by holding the animal gently in the bend of the arm (Meyer and Yost, 1939). On restraint, after the initial struggle had subsided and the animal seemed to be quiet, we still noted heart rates of well over 500 beats per minute. Anesthetics induce sleep but do not produce basal heart rates. A thyroidectomized rat having a resting basal heart rate of 230, gave on application of a needle pick-up through the skin during ether narcosis an initial reading of 350, which declined within 10 minutes to 280, and after 20 minutes to 200. With nembutal anesthesia we obtained readings from 265 to 180 beats. This observation, that, depending on length and depth of anesthesia, any

CHART I. Metabolic rates after thyroidectomy and after thyroid medication.

reading may be obtained is in harmony with the work of many other investigators (Gellhorn, 1943), who reported that anesthesia causes first a sympathetic stimulation that subsides as the anesthesia is deepened and is extended to the lower centers of the brain. It is, therefore, not surprising that our figures differ from those reported by Leblond and Hoff (1944), who used both restraint and anesthesia. The importance of regard for the animals' psychic conditions has also been pointed out by Moses (1944).

When the rats showed consistently athyroid metabolic and heart rates each received for three days powdered thyroid substance containing 0.55 per cent of iodine. This preparation was known to be fully potent as a stimulator of metabolism and to be of a moderate action on the heart rate. Both functions were measured again on the fifth day.

RESULTS

In Chart I are presented the curves of metabolism of 5 rats thyroidectomized on the day corresponding to line zero. The metabolic rates, expressed in mg. O_2 per hour per kg., had previously been determined and correspond to the starting points of the curves. The following determinations were made on the same day of the week for 5 weeks. Line 6 represents either the determination after 6 weeks or the

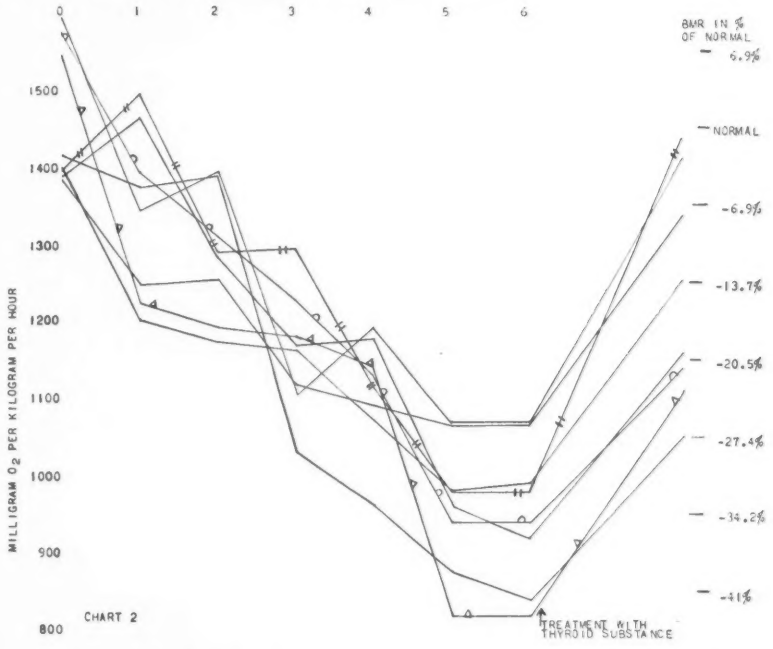

CHART II. Metabolic rate of rats on continuous treatment with thiouracil.

last one made preceding thyroid medication. The dose of thyroid given was the minimum that, under conditions described, will increase the metabolic rate of the thyroidectomized rat to approximately normal levels. This dose had been determined in a large number of other experiments and was reconfirmed in the present tests. The responses are given at the end points of the curves. The time interval is here, however, not 2 weeks as it might appear from the chart, but only 5 days. If the average B.M.R. of 1460 for the normal rat is taken as zero point, the metabolic rates after treatment range between plus and minus 7.0 per cent.

It may be mentioned that for comparative tests it is desirable to use a slightly smaller dosage to keep the metabolic response below that of normal level. Since the effect of increasing thyroid medication

follows a logarithmic curve, the plateau of which corresponds approximately to the B.M.R. of the normal rat, doses higher than the one used will generally give the same response. This fact should be kept in mind if various preparations are to be compared. Varying over-liminal doses will always give identical maxima and the unwarranted conclusion may be drawn that the products used are equally potent. This

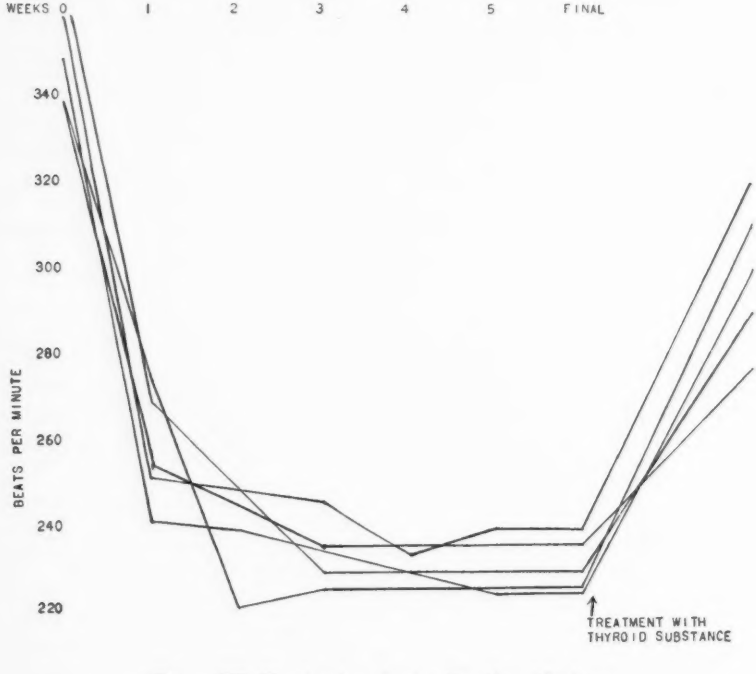

CHART III. Heart rates of rats after thyroidectomy.

criticism may be applied to the publication of McClendon, Foster, and Cavett (1941).

Chart II. shows an analogous presentation of the metabolic rates of 8 rats placed on thiouracil treatment on day zero.

Chart III gives the heart rates of the thyroidectomized animals corresponding to Chart I, and Chart IV those to Chart II. The difference between the corresponding curve groups is quite evident. The rats treated with thiouracil do not show as abrupt a decrease in metabolic rate as do the thyroidectomized animals. This is probably due to the fact that the thyroid contains formed hormone stored in the acini that is not removed as in thyroidectomy, but must first be consumed, although the gland does not produce new hormone. This slow reduction in metabolic rate is in agreement with the report by Rei-

necke, Mixner, and Turner (1945), who measured metabolic rates two weeks after the beginning of thiouracil treatment and found a 23.7 per cent decline. However, their average value for the normal rat of 286.4 mg. CO_2 per 100 g. per hour, equal to 209 mg. O_2, is considerably higher than the average found in our work; namely, 1460 mg. O_2

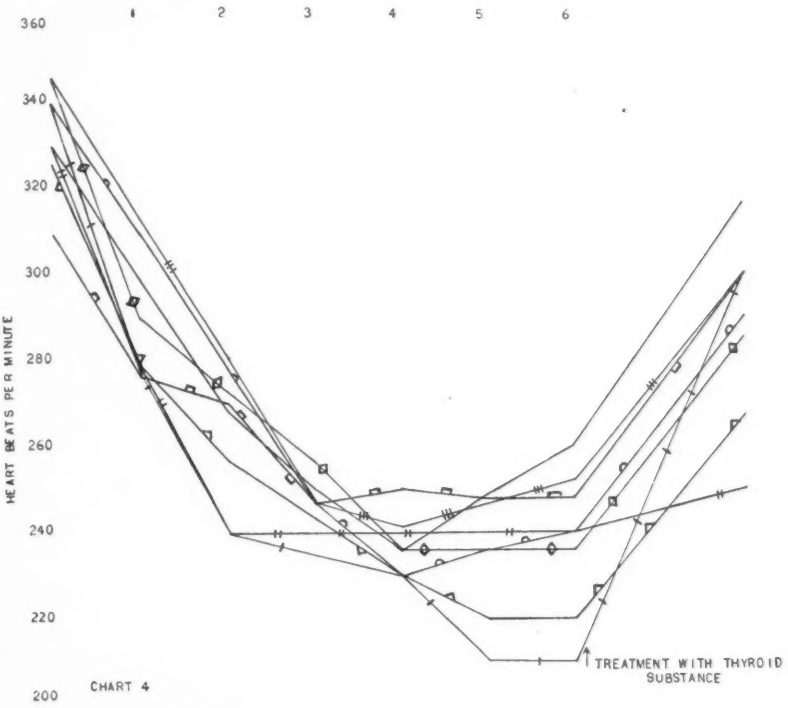

CHART IV. Heart rates of rats on continuous treatment with thiouracil.

per hour per kg. Continued observation showed that the thiouracil rats finally obtained metabolic rates corresponding to the athyroid level. Values below 900 are found in the thyroidectomized rats at an incidence rate of perhaps 5.0 per cent. The fact that 2 out of 8 thiouracil rats had metabolic rates below 900 may be of significance, but the total number is too small to be of statistical value.

While the wider spread of the thiouracil curves is perhaps not absolutely convincing of a lower uniformity as compared with the athyroid group, the differences in response to treatment with thyroid substance are far more striking. The increase in metabolic rate in the thiouracil group after feeding the standard dose is quite erratic and no bioassay could be based on the responses obtained. The variability

may be explained by the assumption that the drug causes other changes in the organism besides the paralyzation of thyroid-hormone production. Perhaps, when deprived of food, some rats do not drink either, which would result in a temporary suspension of the medication.

The heart rate reduction during the first week is in the thyroidectomized group quite uniform and there is little further decrease during the following week. The variations among the individual rats are not significant. As mentioned, the thyroid substance given had been tested before and found to have a moderate but quite definite action on heart rate. The increase in heart rate obtained by this treatment lags slightly as compared with the metabolic response but is equally uniform.

The heart rate curves in the thiouracil rats show a decided analogy with the curves of metabolic rate in that the descent is relatively

TABLE I. DATA OBTAINED BEFORE AND AFTER THYROIDECTOMY

	Before	After							
No. of Weeks		1	2	3	4	5	6–9	10	11
Heart Rate	360	255	240	240	238				270
B. M. R.	1380	1390	1365	1420	1370	1260	1300 av.	1400	1460

Data Obtained with Thiouracil Treatment

No. of Weeks	1	2	4	5	After Thyroid Treatment
Heart Rate	228		242	240	300
B. M. R.	1200	1255	940	1000	1415

slow, and that there is a wide spread between the minima obtained; the reaction to treatment is even more irregular. It appears, then, that in both groups the heart rate and metabolism run approximately parallel. The present investigation was not intended as a continuation of our previous work on metabolism-heart rate relationship and the results cannot be interpreted on the basis of this work.

The expectation that thiouracil would be efficacious even in rats in which thyroidectomy had failed to produce the expected changes was confirmed in one case. A thyroidectomized rat did not develop hypometabolism and was subsequently placed on thiouracil diet, with results presented in Table I.

It may be concluded from these experiments that thiouracil, by acting on any thyroid tissue in the organism, irrespective of location, is more reliable in producing reduction in metabolic rate than thyroidectomy. However, considering the less uniform degree of this effect and the still more variable response to medication with thyroid material, it appears that the thiouracil treated rat is not a satisfactory substitute for the thyroidectomized rat in the bioassay of thyroid material.

SUMMARY

Rats placed on continual treatment with thiouracil given in the diet and drinking water show a slower decline in metabolic and heart rates than do thyroidectomized rats. This is probably due to the presence of stored thyroid hormone in the gland. The final metabolic rates in the thiouracil rats, however, reach athyroid levels. The drug is effective also in animals which, due to accessory thyroid tissue, show no reduced B.M.R. after thyroidectomy. The individual variations in the thiouracil treated rats are considerably larger than in the thyroidectomized animals, and their responses to ingestion of standard doses of thyroid are rather erratic and irregular.

REFERENCES

GELLHORN, E.: *Autonomic Regulations.* pp. 274–277. Interscience Publishers, Inc., New York, 1943.
LEBLOND, C. P., AND H. E. HOFF: *Am. J. Physiol.* 141: 32. 1944.
MCCLENDEN, J. F., W. C. FOSTER AND J. W. CAVETT: *Endocrinology* 29: 928. 1941.
MEYER, A. E., AND A. WERTZ: *Endocrinology* 24: 683. 1939.
MEYER, A. E., AND M. YOST: *Endocrinology* 24: 806. 1939.
MOSES, L. E.: *Am. J. Physiol.* 142: 686. 1944.
REINECKE, E. P., J. P. MIXNER AND C. W. TURNER: *Endocrinology* 36: 64. 1945.

VARIATIONS IN THE WATER CONTENT OF THE RAT'S UTERUS DURING CONTINUOUS ESTROGENIC TREATMENT[1]

WILLIAM R. CARROLL

Captain, Sanitary Corps, Army of the United States[2]

ONE OF THE MOST STRIKING changes occurring in the uterus as a result of treatment with estrogen is the large and relatively rapid increase in water content. This was first discovered by Astwood (1938a) in immature rats and later made the basis of an assay method for determining very small amounts of estrogen (Astwood, 1938b). Periodic uterine edema was demonstrated by Astwood (1939) in the normal estrous cycle of the mature rat. These changes were shown to coincide with the secretion of estrogen—or the release of progesterone, which had been demonstrated to inhibit the water-increasing action of estrogen. However, former investigations were carried out on intact adult rats or on immature rats subjected only to single, short-acting doses of estrogenic hormone. The study reported here was undertaken to furnish a description of the changes in water content of the rat's uterus after castration and during long continuous treatment with estrogen. This allows analysis of the changes in water content of the normal, adult tissue uncomplicated by other ovarian hormones.

METHODS

More than 100 mature, virgin, albino rats weighing 140–220 grams were castrated and after showing diestrous smears for at least two weeks were injected subcutaneously with 50 micrograms of estradiol dipropionate in 0.1 ml. sesame oil. Miescher, Scholz, and Tschopp (1938) have shown that this amount of the diester gives a continuous estrous effect for at least three weeks, and tests made on the strain of rats in the Harvard laboratory showed a similar length of action. At appropriate intervals after injection the rats were killed by a blow on the head; the uteri were removed quickly, split longitudinally, weighed rapidly on an air damped balance to 1 mg. and dried to constant weight at 100–110° C.

Attempts were also made to separate the endometrium from the muscle, thus making it possible to locate more exactly the actual seat of the water imbibition. Such separation of tissues could not be made satisfactorily on uteri earlier than 24 hours after injection because of the undeveloped endo-

Received for publication February 12, 1945.

[1] The data reported here were taken from a thesis submitted to the Department of Biology, Harvard University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Formerly University Fellow, Harvard University, Department of Biology, and Swarthmore Sigma Xi Fellow, 1941–42.

metrial layer. The uteri were first weighed, and then the layers separated in a warm, moist chamber by pulling with forceps. The endometrium and muscle were then weighed separately to check against water loss during the operation and each dried to constant weight. Pieces of skin (from the belly), skeletal muscle, and liver were also removed, weighed, and dried.

A small amount of tissue is unavoidably lost during the separation of endometrium and muscle; consequently, the weighted averages of the separate dry weights of endometrium and muscle do not give exactly the same values as the whole uterus. However, the error is relatively small and con-

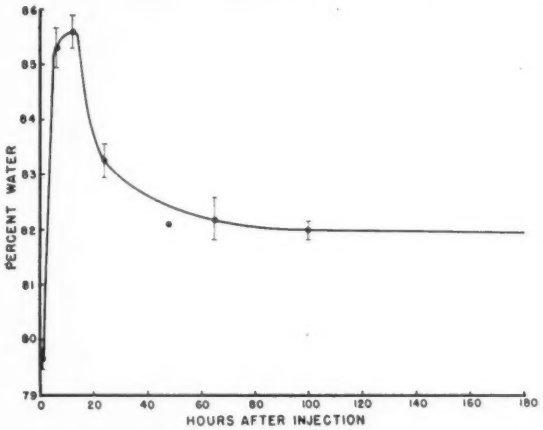

FIG. 1. Changes in the water content of rat uteri during continuous stimulation by estrogen. Each point shown is the average of from 6 to 11 separate determinations. The vertical lines through each point represent twice the standard deviation of the mean. The value given at 100 hours after injection is the average of all points from 80 to more than 200 hours, during which time there is no change.

stant, and the changes reported result after the standard treatment described above.

Stained histological sections showed that during the separation of the endometrial and muscular layers, some circular muscle comes off with the endometrium, but the amount is small and probably negligible.

RESULTS

General: Under the treatment described, the first signs of proestrus appear in the vaginal smear about 40 hours after injection. Full estrus is reached in 72 hours and continues for several weeks. Uteri grow rapidly from the dormant castrate state when they weigh only about 30 mg per horn to organs of more than 150 mg three or four days after injection. During the period from 48 to 80 hours the uterus is distended by a clear fluid filling the lumen, but this gradually disappears. Reinjection does not restore the distention, or modify the water content of the uterus. After 100 hours of treatment the uterus has prac-

tically reached its maximum size, though a small amount of growth does continue for several weeks.

Changes in water content: During the first 40 hours of such administration of estrogen, the changes in water content of the uterus are very similar to those occurring after a single, rapidly absorbed dose in immature animals (see Astwood, 1938b). A rapid influx of water, almost completed within six hours, increases the water content from 79.6 per cent to 85.3 per cent. This typical response to estrogen

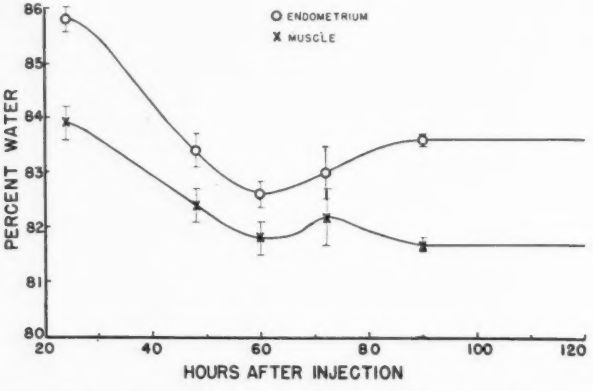

FIG. 2. Changes in the water content of endometrium and muscle of rat uteri during continuous stimulation by estrogen. Other conditions as in legend of Figure 1.

is followed by a slower return to an intermediate level of 82 per cent (Figure 1). In the case of the single injections, as reported by Astwood, the water content returns to normal in about 50 hours, while with the continuous treatment, the water content remains at the intermediate level as long as the estrous effect of the injection lasts, which is at least three weeks.

Corresponding changes in water content take place in both the endometrium and the muscle of the uterus (Figure 2). These changes can only be determined indirectly during the first 20 hours of the action of the hormone, but it is likely that they follow closely the pattern demonstrated for the whole uterus. The similarity in the curves for separate endometrium and muscle after 24 hours indicates that there are no qualitative differences in the reactions of these two kinds of tissue to the hormone.

As has usually been assumed, estrogen produces no changes in the hydration of several other tissues. The water content of both liver and skeletal muscle from rats treated for various lengths of time with estrogen is shown in Table I.

However, Ludden, *et al.* (1941) have reported an increase in the water content of the kidneys of rats during treatment with estrogen,

and Zuckerman, *et al.* (1939) found a series of complimentary shifts in water between genital and non-genital tissues of immature rats after a single injection of estradiol. The possibility that skin, being slightly more reactive to sex hormones in some animals, might show changes in water content was investigated briefly. The few determinations made so far suggest that significant changes may be occurring there.

TABLE I. EFFECT OF CONTINUOUS ESTROGEN TREATMENT ON THE WATER CONTENT OF LIVER AND SKELETAL MUSCLE

Hours after injection	Per cent Water	
	Liver	Muscle
0	71.1	76.6
	68.1	76.3
		75.7
6	69.8	76.4
		76.3
		76.0
12	69.6	75.0
	69.1	75.0
		75.3
24	69.4	76.1
		76.1
48	69.9	76.2
		75.5
60	70.8	76.2
74	68.6	76.7
		75.9
96	71.4	75.7
		75.5
Over 100	69.0	75.0
	68.8	74.8
	71.8	75.2
	69.0	74.7
		77.4
		75.4
		74.7

It is probable that this phenomenon may be demonstrated more clearly if account is made of the large variations in fat content of the skin of rats.

DISCUSSION

From the results reported above it can be seen that a continuous treatment with estrogen causes two successive changes in the water content of the uterus. The first, an excessive edema, is due to a large and rapid influx of both intra- and extra-cellular water (Talbot, Lowry, and Astwood, 1940), which reaches its height about six hours after injection. This is several hours before cell division starts, and

earlier than an increase in oxygen consumption can be demonstrated (Carroll, 1942). As cell division and growth begin, however, the second change in hydration becomes apparent. This involves a gradual decrease in water which reaches a level intermediate between the value for uteri from control animals and from rats six hours after injection. That the decrease is not due to a lack of estrogen was demonstrated repeatedly by reinjections which had no influence on the water content of the uterus. Thus the final level can be considered an equilibrium state reflecting a strongly edematous effect of the hormone being opposed by compensatory mechanisms of the tissues.

The failure of estrogen markedly to influence the water content of other bodily tissues emphasizes the specific action of the hormone on genital tissues.

The general pattern of tissue response following sex hormone stimulation seems always to include a rapid imbibition of water preceding and, in a milder degree, accompanying mitotic activity and growth. For instance, Kosin and Munro (1942), using young male chicks, found this pattern to be followed whether the end organs were combs stimulated by androgen or cloacae stimulated by estrogen. The uterus, vagina, cervix and mammary glands all respond to injections of estrogens by increased mitotic activity and growth, and it is probable that in each instance this response is preceded by at least some degree of hyperemia and edema (Zuckerman, *et al.*, 1939).[3] However, the exact relation of the hydration to growth is obscure. The first sign of growth in the uterus (cell division) appears after the edema has begun to subside; that is, the peak, of mitotic activity occurs several hours later than the high point of water inbibition. Moreover, edema *per se* is not the cause of the increased growth, since it is possible to produce artificial edema not followed by growth (Hechter, Lev, and Soskin, 1940). Thus estrogen produces an increase in water content and also a growth response, but there is no indication that these two phenomena are primarily related. However, the present experiments show that as long as the estrous effect of the injections lasts, the water content of the uterus remains above normal. This indicates that as long as the hormone is stimulating growth it also maintains a relative edema which is probably favorable for growth, as is indicated by the fact that a high water content is characteristic of rapidly growing (embryonic) tissues.

---

[3] The swelling and edema in the "sexual skin" of monkeys that result from the action of estrogen suggest a typical genital tissue response. However, no increase in mitotic activity has been demonstrated to follow, and the edema is a peculiar type, the subepithelial connective tissue holding most of the water in a form more like Wharton's jelly of the umbilical cord than the usual intercellular plasma filtrate. This seems to indicate a type of reaction different than that of the uterus and other genital tissues.

CONCLUSIONS

Continuous stimulation with estrogen causes a rapid increase in the water content of the rat's uterus followed by a drop to an intermediate level, which is maintained indefinitely. Changes in hydration of uterine muscle and endometrium differ only in degree, endometrium having a greater degree of hydration throughout. The water content of skeletal muscle and liver is not affected by estrogen treatment.

REFERENCES

ASTWOOD, E. B.: *Anat. Rec.* 20 (supplement): 5. 1938a; *Endocrinology* 23: 25. 1938b; *Am. J. Physiol.* 126: 162. 1939.

CARROLL, W. R.: *Proc. Soc. Exper. Biol. & Med.* 49: 50. 1942.

HECHTER, OSCAR, MAURICE LEV AND SAMUEL SOSKIN: *Endocrinology* 26: 73. 1940.

KOSIN, IGOR L., AND S. S. MUNRO: *Endocrinology* 30: 102. 1942.

LUDDEN, JOHN B., ERICH KREUGER AND IRVING S. WRIGHT: *Endocrinology* 28: 619. 1941.

MIESCHER, K., C. SCHOLZ AND E. TSCHOPP: *Biochem. J.* 32: 725. 1938.

TALBOT, N. B., O. H. LOWRY AND E. B. ASTWOOD: *J. Biol. Chem.* 132: 1. 1940.

ZUCKERMAN, S., A. PALMER AND G. BOURNE: *Nature, London* 143: 521. 1939.

THE RESPONSE TO THYROXINE AFTER SUBTOTAL HEPATECTOMY[1]

PETER E. KELLAWAY, HEBBEL E. HOFF AND CHARLES P. LEBLOND

From the Departments of Physiology and Anatomy, McGill University

MONTREAL, CANADA

WHEN THYROXINE is injected into an animal and the bile assayed, definite amounts of iodine may be detected, amounting in some experiments to an excretion within three hours after injection of as much as 47 per cent of the total quantity administered (Kendall 1919, Krayer 1928). Bioassay (by the method of axolotl metamorphosis) indicates that, while some of the iodine is present in an hormonally active form, there has been considerable destruction of the administered hormone (Krayer 1928). Chemical fractionation methods, on the other hand, indicate that as much as one-half to two-thirds of the thyroxine may be excreted unchanged (Barnes 1933, Zawadowsky & Perelmutter 1927).

It appears from these experiments that the liver is able either to excrete thyroxine in the bile, or to destroy the hormone and excrete the iodine in a physiologically inactive form. Which of these two courses is taken apparently depends upon the magnitude of the thyroxine concentration with which the liver is presented. It is less apparent, though very likely, that the natural endogenous hormone when present in excess is similarly decomposed and excreted (Salter 1940).

The experiments to be described were designed to clarify further the function of the liver in thyroxine metabolism, and concern fundamentally the assay of hormonal activity in animals in which decomposition or excretion of administered thyroxine or endogenous thyroid hormone was influenced by subtotal hepatectomy or ligation of the bile duct. The heart rate was employed as a measure of thyroxine activity because the action of this substance upon the myocardium has been shown to be a direct one (Lewis & McEachern 1931, Priestley *et al.* 1931, Yater 1931, Markowitz & Yater 1932), and because it provides a convenient, consistent and rapid method of assay, entailing little disturbance of the animal.

METHODS

A preliminary study was made to determine the factors which influence the heart rate in the rat, and to determine the normal rate and the degree of

Received for publication January 29, 1945.

[1] These investigations were supported by a grant from the Blanche Hutchinson Fund of the Faculty of Medicine, McGill University.

variation from the norm under control conditions. Time of day, environmental temperature, diet, general handling of the animals, and the surrounding noise level were all found to influence the heart rate. The individual heart rate varies at different times of the day, being slow in the morning and slowing more toward noon, at which level it remains until late afternoon, but as evening is approached it rises and reaches a maximum in the first few hours of the morning. However, other factors being equal, we found that if the electrocardiograms were taken at the same time (4:30 p.m.) each day individual and group variation was very small.

Environmental temperature is important in that we observed that an increase in temperature had the effect of slightly depressing the rate in normal animals and slightly increasing it in thyroidectomized animals. Care was therefore taken to prevent significant variations in the temperature of the room where the animals were kept, and where the electrocardiograms were taken. Although the effect of diet was not studied systematically, it was observed that animals deprived of food or water, or of both, were highly excitable and the heart rate was much influenced by handling. Even under normal conditions, loud or sudden sounds tend to frighten the animals and may therefore influence the heart rate. Furthermore, a noisy room makes the animals restless and difficult to handle.

Before using the rats in the experiments, an effort was made to accustom them to handling, and during each experiment we were careful to pick them up gently and to avoid sudden movements.

When these precautions were taken, the heart rate did not vary more than ± 15 beats/min. in normal animals and ± 10 beats/min. in thyroidectomized animals. Similarly in both thyroidectomized and intact groups the greatest individual variations from the mean were never more than ± 20 beats/min. when the animals were healthy. In a series of 160 recordings of the heart rate in 32 normal individuals, the average rate was 436 beats/min., the highest rate being 454 beats/min. and the lowest, 409 beats/min. The variation among thyroidectomized rats was even smaller. The mean for four hundred records of the rate in 110 thyroidectomized rats was 323 beats/min. with a maximum recording of 343 beats/min. and a minimum of 310 beats/min.

Procedure. Stainless-steel wire electrodes were sewn into the skin of the chest wall, one caudal and slightly ventral to each shoulder joint. The electrodes were so-placed because the animals were less able to pull them out here than at any other suitable point. When a record was to be taken, a very small clip was attached to each electrode. These in turn were connected by 33-gauge silk-covered wire to the terminals of Lead I of a Sanborn Cardiette. Thus connected, the animals were placed on a wooden fork (hind feet on one prong and front feet on the other) which was attached to the top of an apparatus stand 52 inches above the ground. This fork was first employed by Marcuse and Moore (1941) who found that the animals remained remarkably still during the time necessary for taking a record.

In all the experiments described here female albino rats weighing 100 gm. ± 10 gm. were employed. All operations were done under ether anesthesia and the thyroidectomized rats were not used until three weeks after operation.

Experiment 1. Forty rats were divided into 4 groups of 10, as follows:

group A—thyroidectomized; group B—thyroidectomized, hepatectomized; group C—thyroidectomized, thyroxine-treated; group D—thyroidectomized, hepatectomized, thyroxine-treated.

The partial hepatectomy in groups B and D was done under ether by a method which removes 85 per cent by weight of liver tissue (Selye et al 1936). Thyroxine dosage was begun immediately after the operation, 7.5 μg. being injected subcutaneously twice a day for the duration of the experiment. To avoid complicating the autopsy findings, the experiment was terminated after 11 days, as previous observation had shown that by this time the liver is completely regenerated as far as weight and size are concerned. The heart rate was measured every 48 hours. The metabolic rate was measured in all groups on the 8th day by the method of Williams, Phelps, and Burch (1941).

Experiment 2. Two groups of 10 animals were employed: group A—normal controls; group B—hepatectomized. Electrocardiograms were taken every 48 hours for 10 days.

Experiment 3. Two groups of 8 animals were employed: group A—thyroidectomized, thyroxine-treated; group B—thyroidectomized, bile duct ligated, thyroxine-treated. Three weeks after thyroidectomy a ligature was tied around each tributary bile duct and around the proximal and distal ends of the ductus choledochus. The part of the latter duct between the ligatures was then removed. This procedure produced a complete obstruction of bile flow, and clear signs of jaundice appeared by the 2nd day (accessory ducts did not develop).

Immediately after operation thyroxine dosage was begun, two subcutaneous injections of 7.5 μg. being administered daily. Electrocardiograms were taken for each animal every 2nd day and the rates compared with those of a group of thyroidectomized controls receiving the same thyroxine dosage.

Experiment 4. Two groups of 6 animals were used: group A—thyroidectomized, thyroxine-treated; group B—thyroidectomized, hepatectomized, thyroxine-treated. This experiment is essentially the same as experiment 1 with the exception that the thyroxine dosage was reduced to two daily subcutaneous injections of 2.5 μg. Electrocardiograms were taken on the 2nd, 5th, and 8th days after hepatectomy.

Experiment 5. Three groups of 8 animals were employed: group A—thyroidectomized, thyrotropin-treated; group B—normal, thyrotropin-treated; group C—hepatectomized, thyrotropin-treated. On the day group C was hepatectomized, all groups were given 3 subcutaneous injections of 1/3 cc. of thyrotropic hormone[2] and similar doses on the following 2 days. Electrocardiograms were taken every 24 hours.

RESULTS

The results of experiment 1 are shown in Figure 1. The heart rate did not vary significantly from day to day in the thyroidectomized group. Likewise hepatectomized, thyroidectomized animals did not differ in heart rate from thyroidectomized animals, nor did they show any significant variation throughout the experiment. The average metabolic rate as determined on the 8th day was 120 liters of O_2 per

[2] Antuitrin-T (Parke Davis and Company).

sq. meter per day in the thyroidectomized group and 129 liters per sq. meter per day in the thyroidectomized, hepatectomized group.

Thyroidectomized animals receiving 15 μg. of thyroxine daily showed a gradual increase in heart rate, which finally reached the normal level about the 11th day. The average predosage rate for this group was 333 beats/min. By the 11th day, after a total of 165 μg. of

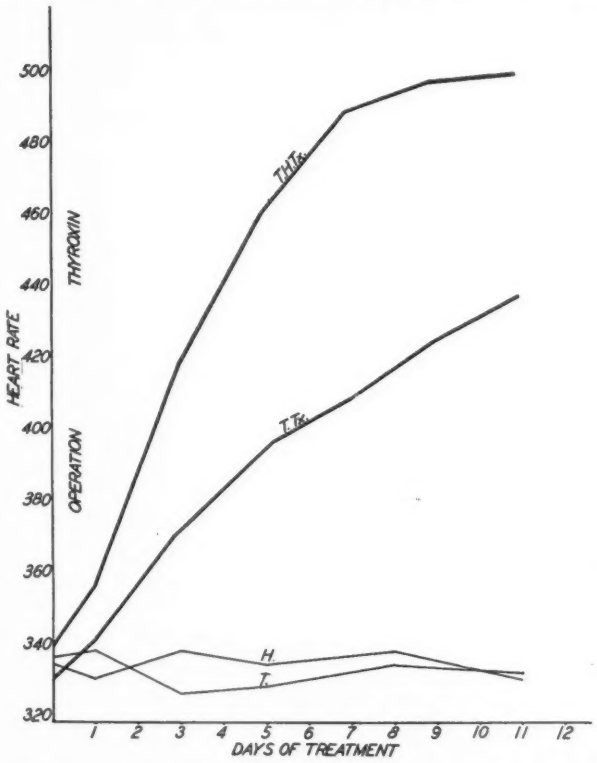

FIG. 1. The average heart rates in the four groups of rats in experiment 1. T = thyroidectomized; TH = thyroidectomized, hepatectomized; TTX = thyroidectomized, thyroxine-treated; THTX = thyroidectomized, hepatectomized, thyroxine-treated.

thyroxine had been administered, the level rose to 438 beats/min., which is approximately the mean for normal animals. The average basal metabolic rate for this group as measured on the 8th day was 188 liters of O_2 per sq. meter per day.

The thyroidectomized, hepatectomized group receiving the same daily dose of thyroxine showed a more abrupt response to treatment and reached the normal level (438 beats/min.) on about the 4th day, when they had received only 60 μg. of thyroxine—less than half the amount required to raise the non-hepatectomized group to the same

level. By the 7th day the average rate had reached 490 beats/min. but after this the rise in rate tended to taper off, and in the next 4 days increased only 10 beats, which may or may not represent an actual rise. This leveling off of the response to treatment is probably due to regeneration of the liver, which proceeds more and more rapidly as the metabolism rises. At autopsy the weight of the liver in this group far exceeded the weight of that organ in the thyroidectomized, thyroxine-treated group (see Table 5). The average basal metabolic rate for this group on the 8th day was 244 liters of O_2 per sq. meter per day.

TABLE 1. COMPARISON OF THE HEART RATE OF NORMAL RATS WITH THAT OF HEPATECTOMIZED RATS SHOWING THE AVERAGE RATE AND RANGE IN EACH GROUP

Group	Preoperative	2nd day	4th day	6th day	8th day
Normal controls	442 (421–453)	428 (404–444)	436 (416–452)	440 (423–453)	446 (415–452)
Hepatectomized	436 (416–448)	441 (420–452)	448 (426–456)	437 (420–447)	450 (428–457)

TABLE 2. COMPARISON OF THE EFFECTS OF 5 μg. OF THYROXINE DAILY ON THYROIDECTOMIZED AND THYROIDECTOMIZED, HEPATECTOMIZED RATS SHOWING THE AVERAGE RATE AND RANGE IN EACH GROUP

Group	Pre-dosage rate	2nd day (5 μg.)	5th day (25 μg.)	8th day (40 μg.)
Thyroidectomized	321 (312–342)	349 (330–353)	360 (340–372)	371 (346–387)
Thyroidectomized, Hepatectomized	334 (318–338)	354 (329–360)	368 (341–379)	379 (350–391)

Normal animals which are hepatectomized do not show any elevation in heart rate, as shown by the results of experiment 3 (see Table 1). This may mean either that the liver does not play a part when the output of the thyroid hormone is within physiologic limits, or that the thyroid hormone as secreted by the gland differs from thyroxine and in this form is not influenced by the liver. That the first of these possibilities is correct is indicated by the fact that when a maintaining dose of thyroxine, namely, 5 μg. daily, was administered instead of the 15 μg. used in experiment 1, there was no significant difference in pulse rate between the hepatectomized, thyroidectomized group and the thyroidectomized controls receiving the same dosage (experiment 4—Table 2). Furthermore, when equal doses of thyrotropic hormone were administered to thyroidectomized, to hepatectomized, and to intact animals, the hepatectomized group exhibited a far greater response than the intact animals, the heart rate rising from 429–515 beats/min. in the former and from 435–462 beats/min. in the latter. The average heart rate for the thyroidectomized group

was uninfluenced by the thyrotropic hormone, showing that the effect on the heart in the other two groups was not a direct response to the substance itself but probably due to the increased secretion by the thyroid gland (Table 3).

TABLE 3. THE RESPONSES OF NORMAL, HEPATECTOMIZED, AND THYROIDECTOMIZED RATS TO THYROTROPIC HORMONE (1/3 CC. ADMINISTERED THRICE DAILY FOR 3 DAYS)

Group	Pre-dosage rate	After 1 cc.	After 2 cc.	After 3 cc.
Normal	435 (410–451)	430 (418–449)	442 (424–450)	462 (439–478)
Hepatectomized	429 (411–448)	440 (419–461)	478 (460–496)	515 (489–538)
Thyroidectomized	335 (312–346)	329 (316–347)	331 (318–342)	332 (310–346)

TABLE 4. COMPARISON OF THE EFFECTS OF THYROXINE (15 μg. DAILY) ON THE HEART RATE OF THYROIDECTOMIZED RATS AND THYROIDECTOMIZED RATS WITH BILE DUCT LIGATED

Group	Pre-dosage	15 μg.	45 μg.	75 μg.	105 μg.	135 μg.
Thyroidectomized	326 (312–338)	338 (320–345)	362 (336–371)	395 (368–406)	418 (390–432)	432 (401–462)
Thyroidectomized, Bile duct ligated	323 (310–340)	330 (318–340)	346 (330–354)	372 (339–398)	391 (342–426)	410 (349–461)

TABLE 5. ORGAN WEIGHTS OF RATS IN THE VARIOUS GROUPS OF EXPERIMENT 1

Organ	Thyroidectomized	Thyroidectomized hepatectomized	Thyroidectomized thyroxine-treated	Thyroidectomized hepatectomized thyroxine-treated
Adrenal	34.75 mg.	47.8 mg.	49.9 mg.	56.8 mg.
Ovary	27.2 mg.	37.8 mg.	36.6 mg.	50.9 mg.
Uterus	190.0 mg.	209.0 mg.	260.0 mg.	300.0 mg.
Heart	544.0 mg.	530.0 mg.	652.0 mg.	681.0 mg.
Spleen	488.0 mg.	829.0 mg.	1109.0 mg.	781.0 mg.
Liver	5431.0 mg.	5020.0 mg.	5628.0 mg.	7555.0 mg.
Kidney	1160.0 mg.	1367.0 mg.	1720.0 mg.	1528.0 mg.
Pituitary	—	12.6 mg.	11.4 mg.	10.8 mg.

The response of thyroidectomized animals to thyroxine treatment is not increased after ligation of the bile duct. This is indicated by the fact that, when equal doses of thyroxine were administered to thyroidectomized rats in which the bile ducts were ligated and to thyroidectomized controls, the former group did not show a greater increase in heart rate than the control animals (Table 4). It seems clear therefore that at the dosage employed thyroxine as such is not excreted via the bile.

XUM

DISCUSSION

The results of experiment 1 show very clearly that the activity of thyroxine as measured by the basal metabolic and pulse rates is greatly intensified after partial hepatectomy when injected in excess of the normal requirement. When, however, thyroxine dosage is kept at a physiologic or maintaining level (5 μg. daily), the intensification of the response after partial extirpation of the liver is not manifest: either it does not occur at this level or it is not demonstrable by the technics employed.

The thyroid hormone, when secreted by the gland in excess, is more active if liver function is impaired. This is demonstrated by the fact that, when equal doses of thyrotropic hormone were administered to normal and to hepatectomized rats, the latter showed a much greater elevation in pulse rate than the former. A measurable intensification of hormone activity does not occur after liver extirpation when the output of the gland is within normal limits (experiment 2).

Previous experience in this laboratory (Leblond & Hoff 1944) has indicated that the maintaining dose of thyroxine for the thyroidectomized 100 gm. rat is approximately 5 μg., and Dempsey and Astwood (1943) have calculated that at the environmental temperature in which the animals used in these experiments were kept, namely 75° F., this represents the approximate daily output of the normal gland. When such a dose of thyroxine was injected into thyroidectomized and thyroidectomized, hepatectomized animals for a period of 8 days, there was no significant difference in the average rate for the two groups.

This finding is compatible with the observations of Kendall (1910) who found that under normal conditions a dynamic equilibrium exists between the thyroxine in the thyroid gland, the amount in the blood, and the amount in the tissues. The suggestion is that under physiologic conditions the tissues absorb only their normal requirement of hormone and that a balance exists between the demand on the gland and its output. These experiments are taken as evidence, therefore, that the liver is not required to play a part when the amount of circulating thyroid hormone is within the limits of the body's requirements, but tends to maintain the equilibrium between thyroid, blood, and tissues by suppressing the activity of the hormone when it is present in excess of the tissue's demands.

Kendall (1919) injected 200 mg. of thyroxine into the saphenous vein of a dog and found that 43 per cent of the total iodine contained in the thyroxine injected was excreted in the bile within the next 50 hours. Krayer (1928) obtained similar results in the rat. If this iodine is of hormonal origin, it probably represents a breakdown product of the injected thyroxine, because the animals in experiment 3 in which the bile duct was ligated did not show greater increase in heart rate

in response to thyroxine medication than did control animals receiving the same dose. Obviously, thyroxine is not excreted functionally intact but must undergo some change in the liver which suppresses its activity. Just what this change is cannot be said at this time but several observations should be mentioned in this connection. Zawadowsky and Perelmutter (1927) found that the decomposition and excretion of thyroxine is a function of the liver in the domestic fowl. Similarly two Japanese investigators (Horiuti & Ohsako 1934) claim that "yakriton," the reputed detoxicating hormone of the liver, counteracts the effect of thyroid hormone on the metamorphosis of tadpoles. The work of Felix and Zorn (1941) is also suggestive: they found that, when tyrosine is incubated with liver pulp, decomposition of the molecule occurs.

It should be pointed out that there is an alternative explanation for the above experimental findings, namely, that subtotal hepatectomy alters the metabolism of the heart in such a way that its sensitivity to thyroxine increases. Such an explanation would not account, however, for the failure of hepatectomy to increase the heart rate in normal rats and in thyroidectomized rats receiving small doses of thyroxine.

SUMMARY

A study was made of thyro-hepatic interrelationships, the heart rate being used as a measure of thyroxine activity. It was found (1) that thyroxine activity is greatly intensified in the absence of the liver; (2) that the liver does not play a significant role when the amount of circulating thyroxine is within physiologic limits; (3) that the liver deals with excess hormone by some process of inactivation and not by simple excretion.

REFERENCES

Barnes, B. O.: *Am. J. Physiol.* 103: 699. 1933.
Dempsey, E. W., and E. B. Astwood: *Endocrinology* 32: 509. 1943.
Felix, K., and K. Zorn: *Ztschr. f. physiol. Chem.* 268: 257. 1941.
Horiuti, I., and H. Ohsako: *Tohoku J. Exper. Med.* 24: 505. 1934.
Kendall, E. P.: *Endocrinology* 3: 156. 1919.
Krayer, O.: *Arch. f. exper. Path. u. Pharmakol.* 128: 116. 1928.
Lewis, J. K., and D. McEachern: *Proc. Soc. Exper. Biol. & Med.* 28: 504. 1931.
Marcuse, F., and A. U. Moore: *Proc. Soc. Exper. Biol. & Med.* 48: 201. 1941.
Markowitz, C., and W. M. Yater: *Am. J. Physiol.* 100: 162. 1932.
Priestley, J. T., J. Markowitz, and F. C. Mann: *Am. J. Physiol.* 98: 357. 1931.
Salter, W. T.: The Endocrine Function of Iodine. Harvard University Press, Cambridge, 1940.
Selye, H., R. L. Stehle, and J. B. Collip: *Canad. M.A.J.* 34: 339. 1936.
Yater, W. M.: *Am. J. Physiol.* 98: 338. 1931.
Williams, C., D. Phelps, and J. C. Burch: *Endocrinology* 28: 816. 1941.
Zawadowsky, B. M., and Z. M. Perelmutter: *Arch. f. Entwcklngs-mechn. d. Organ.* 109: 211. 1927.

XUM

NOTES AND COMMENTS

THE EFFECT OF GROWTH HORMONE ON THE VITAMIN A-DEFICIENT RAT

ALTHOUGH it can readily be demonstrated that administration of growth hormone to male and female rats exhibiting a "plateau of growth" is followed by an increment in body weight, there is evidence to indicate this effect will not occur if the diet is inadequate for proper nutrition. Simpson and Evans[1] failed to obtain growth in thiamine-deficient rats following administration of an anterior pituitary growth-promoting extract; while Margitay-Becht and Wallner (1937) reported similar results for animals deficient in vitamin A. In the present communication further data is presented on the effects of "growth hormone" administration to animals deficient in vitamin A.

EXPERIMENTAL

The animals employed in the present experiment were rats of the University of Southern California strain in which the dietary regime of the parents was such as to make the young rats satisfactory for bioassay studies on vitamin A. Parents were maintained for at least two months previous to breeding and for 14 days after the birth of the litter on Sherman diet B (Campbell and Sherman, 1924) without addition of supplementary lettuce or meat. Litters were cut to seven at three days of age, and on the fourteenth day mothers and litters were placed on a vitamin A-low diet.[2] After weaning, the young rats were placed on the vitamin A-depletion diet[3] on which they were maintained for the duration of the experiment. Two series of experiments were carried out: (1) in which growth hormone was administered after animals had plateaued in body weight and (2) in which growth hormone was administered from the day animals were placed on the vitamin A-depletion diet.

In series I rats were weaned between 21 and 27 days when body weight averaged 40–50 grams. After vitamin A-deficiency symptoms were noted such as stationary or decreasing body weight over a five-day period and xerophthalmia, litter mates were divided as far as possible in the five groups and given the supplements as indicated in Table I over the following 28 days or until death if that occurred earlier. In series II animals were weaned at 22 days when their weight averaged 37 grams. Supplements were started immediately and continued for 28 days after body weight plateaued or until death if that occurred earlier. Four groups were employed (Table I). Animals in series I received 0.5 mg. of alpha-tocopherol daily in addition to that

Received for publication January 8, 1945.

[1] Personal communication from Dr. H. M. Evans.

[2] Similar to U.S.P. XII depletion diet except that commercial casein rather than extracted (vitamin A-free) casein is employed.

[3] Obtained from S.M.A. Corporation, Chagrin Falls, Ohio.

TABLE I. THE EFFECT OF GROWTH HORMONE ADMINISTERED WITH OR WITHOUT VITAMIN A ON GROWTH AND ON SURVIVAL TIME OF RATS ON A VITAMIN A-FREE DIET

Group No.	Number of rats		Supplements given daily		Values at end of depletion period		Increase in body weight on following days of assay period[1]						Final weight	Per cent surviving	Average survival time of descendents
	Males	Females	Vitamin A	Growth hormone	Average age	Average wt.	5th	10th	15th	20th	25th	28th			
							Series I—Supplements started after vitamin A depletion (Assay period only).								
			I.U.	*rat units*	*days*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*		*days*
1	4	4	0	0.0[2]	49.1	93.6	−0.1	−3.8	−8.0	−11.0	−14.8 (6)	−14.3 (5)	81.6	62.5	22.1
2	3	3	0	2.5	46.5	87.8	1.8 (5)	−0.7 (4)	−1.6 (3)	−0.7 (3)	−1.5 (2)	−5.0 (2)	86.5	33.3	17.3
3	7	4	0	25.0	49.2	91.2	0.1 (9)	3.8 (5)	5.0 (4)	−8.7 (3)	—	—	—	0.0	11.3
4	3	3	10	0.0[2]	48.2	91.2	11.8	23.6	34.4	45.2	55.2	60.1	151.3	100.0	—
5	3	3	10	25.0	47.5	83.4	14.0	31.6	45.2	59.2	75.2	81.3	164.7	100.0	—
							Series II—Supplements started at weaning and continued throughout.								
6	5	5	0	0.0[2]	45.2	84.5	−1.8	−2.2	−6.6	−3.6 (6)	−10.0 (4)	−10.5 (2)	70.0	20.0	22.5
7	5	5	0	2.5	44.4	92.1	−5.7 (9)	−4.3 (7)	−2.0 (5)	−4.0 (3)	−6.0 (1)	−13.0 (1)	76.0	10.0	13.9
8	4	4	0	25.0	43.0	90.7	−3.0 (6)	−4.0 (5)	−0.6 (5)	−5.0 (2)	—	—	—	0.0	12.4
9[3]	6	4	10	0.0[2]	—	—	—	—	—	—	—	—	184.5	100.0	—

The values in parentheses indicate the number of animals which survived of which this is an average.
[1] Where unequal sex distribution occurred, these values are based on weighting results on males and females equally.
[2] Received injections of placebo intraperitoneally daily.
[3] Since vitamin A was administered throughout, no plateauing occurred and the final weight is the average 8 weeks after the supplement was started.

present in the basal ration; in series II no additional tocopherol was employed.

The growth hormone employed in the present experiment consisted of a powdered extract of the anterior pituitary gland assaying according to Dr. Oliver Kamm, Parke, Davis and Co., 1700 rat (growth) units per gram.[4] This material was dissolved in alkaline aqueous solution,[5] pH adjusted to 8.5, and two concentrations prepared, i.e., 2.5 and 25 rat (growth) units per cc. respectively. Normal butyl alcohol was added in one per cent concentration as a mild preservative. The placebo solution was prepared by an identical procedure but the pituitary extract was omitted. Vitamin A was administered in an amount of 10 I.U. per day.[6]

The results of the tests are summarized in Table I.

DISCUSSION

Results confirm the finding of Margitay-Becht and Wallner (1937) that administration of "growth hormone" to vitamin A-deficient rats failed to promote a "gain in body weight" (groups 2, 3, 7 and 8). Growth, however, was readily obtained following administration of vitamin A, particularly

[4] This was kindly furnished through the courtesy of Dr. Oliver Kamm, Parker Davis and Co., Detroit, Michigan. Unpublished work from this University of Southern California laboratory has confirmed the growth-promoting properties of this extract. Daily intraperitoneal injections of this material to 20 rats for a 12 week period starting at weaning and equivalent to 10 rat (growth) units per day at the stated potency resulted in a consistent gain in body weight from 20 to 60 grams greater than that observed in saline injected controls.

[5] We wish to thank Mrs. C. H. Johnston who prepared these solutions.

[6] Nopco Fish Oil Concentrate (assaying 1,000,000 I.U. per gram) dissolved in ethyl alcohol.

XUM

when administered in conjunction with anterior pituitary extract (group 5). These findings indicate either (1) that tissues of the A-deficient rat are refractory to growth hormone or (2) that the A-deficient rat is lacking some metabolite essential for growth hormone activity.

This is the first report of the deleterious effect of "growth hormone" when administered in the absence of vitamin A. Administration of the pituitary extract to A-deficient rats resulted in an unexpectedly high mortality rate as well as a marked decrease in the average length of survival. Whereas 7 of the 18 negative controls survived the 28 day period (groups 1 and 6), all rats which received the growth hormone at a level of 25 rat units daily without vitamin A died (groups 3 and 8), the average survival time being 12 days. Furthermore, since the average survival time can be based only on decedents and not on the total number, it is obvious that the effect of the growth hormone is greater than the variations in survival time would indicate. Results on rats which received growth hormone at one-tenth this level but also without vitamin A (groups 2 and 7) were intermediate between these extremes both in per cent surviving and in survival time. Symptoms of muscular incoordination, paralysis of the hind limbs, and paresthesia of the skin and tail were likewise precipitated earlier and more pronounced in the rats receiving growth hormone but no vitamin A than in the negative control groups.

We are unable on the basis of the present data to explain the early deaths resulting from administration of the pituitary extract. It is not unlikely that administration of growth hormone to plateaued rats deficient in vitamin A depleted body stores of this vitamin sooner than might otherwise have occurred, precipitating acute symptoms of vitamin A deficiency and early deaths.

Although the growth hormone was not a pure preparation, it would seem improbable that toxic substances caused the exaggerated effects noted when this extract was administered in the absence of vitamin A. Were this the case, one would expect a similar toxicity to be observed in the presence of vitamin A rather than the augmentatory effected noted in group 5. It could be argued, however, that the toxic principles were directly counteracted by vitamin A.

SUMMARY

Administration of a growth-promoting extract of the anterior pituitary failed to promote growth in plateaued rats deficient in vitamin A. Administration of the pituitary material resulted however in an increased mortality rate, a marked decrease in average length of survival and the precipitation of acute symptoms of vitamin A deficiency both earlier and more pronounced than those observed in saline treated controls.

B. H. Ershoff and Harry J. Deuel, Jr.
From the Emory W. Thurston Laboratories, Los Angeles, and the Department of Biochemistry and Nutrition, University of Southern California Medical School, Los Angeles, California

REFERENCES

Margitay-Becht, E., and E. Wallner: *Ztschr. f. Vitaminforsch.* 6: 119. 1937.
Sherman, H. C., and H. L. Campbell: *J. Biol. Chem.* 60: 5. 1924.

ABSORPTION OF SUBCUTANEOUSLY IMPLANTED HORMONE PELLETS

SHIMKIN, LORENZ, WYMAN AND NORTON (1944) have recently shown that the progressive decrease in the absorption rate of cast pellets (i.e. pellets prepared by solidification of fused hormone) of diethylstilboestrol, subcutaneously implanted into mice, can be completely accounted for by the gradual decrease in surface area of the pellets due to absorption. This means (a) that absorption rate at any instant is proportional to surface area and (b) that the observed falling off in absorption rate is not due to encapsulation of the pellets.

It may be of interest to point out, since Shimkin *et al.* do not appear to have seen our paper (Bishop and Folley, 1944) probably because of war-time conditions, that we had previously reported a study of the absorption kinetics of cast pellets of testosterone subcutaneously implanted into humans, which led us to identical conclusions. We showed that out results gave a satisfactory fit to the equation

$$A = 100\left\{1 - \frac{(r-kt)^2(h-2kt)}{r^2h}\right\}$$

where A = the precentage loss in weight at time t of a cylindrical pellet of initial radius r and initial height h, and k = a constant. This equation, which was deduced on the assumption that absorption rate at any instant is proportional to the surface area, can easily be transformed into a form identical with equation (3) of Shimkin *et al.* The results of the latter workers thus provide complete confirmation in mice of our findings in humans.

The above findings apply to cast pellets; with compressed pellets other factors must be taken into account. Thus large compressed pellets of pure hexoestrol show at the outset an abnormally high absorption rate due to the loss of an easily soluble component but thereafter the absorption rate is approximately linear for a considerable period and there is evidence that the absorption rate during the linear phase is proportional to surface area (Folley 1944). How far absorption during the linear phase is affected by the deposition of insoluble protein material in the pores of the tablet (ghost formation; Folley, 1942) it is impossible to say, but it does not affect the situation as regards cast pellets, since as Deanesly & Parkes (1943) have shown, ghost formation does not occur in cast pellets.

P. M. F. BISHOP

Endocrine Clinic, Guy's Hospital,
London, S.E.I., England.

S. J. FOLLEY

National Institute for Research in Dairying,
University of Reading, England

REFERENCES

BISHOP, P. M. F., AND S. J. FOLLEY: *Lancet* i: 434. 1944.
DEANESLY, R., AND A. S. PARKES: *Lancet* ii: 500. 1943.
FOLLEY, S. J.: *Nature, London* 150: 403. 1942.
FOLLEY, S. J.. *Proc. Roy. Soc. London S. B.* 132: 142. 1944.
SHIMKIN, M. B., E. LORENZ, R. WYMAN AND S. G. NORTON: *Endocrinology* 35: 283. 1944.

Received for publication February 8, 1945.

A NOTE ON THE BILIARY EXCRETION OF EXOGENOUS ESTROGEN

CANTAROW and his associates (1942; 1943; 1944) have presented data that suggest that the bile is an important medium of excretion of exogenous and endogenous estrogen. The claim of the existence of an entero-hepatic circulation of estrogen, similar to that of bile acids, is startling when viewed against an apparently overwhelming mass of evidence that estrogens are rapidly inactivated by the liver (literature reviewed by Cantarow *et al.* (1942; 1943). It is significant in this connection that comparatively large amounts of estrogen, in terms of estrogenic activity, have been demonstrated in the bile of various species, including man, as well as in commercial bile-salt preparations and meconium (Gsell-Busse, 1928; 1929).

In an earlier study, Cantarow and his associates (1942) recovered, in the bile of the dog, almost all of the estrogenic activity of intravenously administered estrogen. Longwell and McKee (1942) recovered only 1.3–8.0 per cent of the estrogenic activity in the bile of dogs injected subcutaneously with estrone; the bile estrogens had been purified and fractionated. Since Cantarow and his associates had not performed any chemical studies of the bile estrogens, it was considered essential to repeat previous work in order to correct this deficiency. Accordingly, apha-estradiol was given intravenously, in absolute alcohol, in 10 doses of 10 mg. each, at intervals of 72 hours, to a bile-fistula dog, the bile draining externally into a balloon. The estrogenic activity of each 72-hour sample was determined in the Philadelphia laboratory (Fluhmann technic), 10 per cent of the volume of each specimen being removed for this purpose. The remainder of the pooled bile specimens, preserved with merthiolate, was sent to the Worcester laboratory for chemical study. The results obtained by the Philadelphia and Worcester laboratories are presented in Tables 1 and 2, respectively.

Comment: The average recovery of estrogenic activity in the bile is considerably less than that reported in previous studies (Cantarow *et al.*, 1942), the Philadelphia laboratory recovering 22.4 per cent free estrogen, and 26.4 per cent total, and the Worcester laboratory 10.4 per cent free estrogen. However, in previous experiments, the quantity of estrogen administered ranged from 1 mg. estrone to 2.5 mg. alpha-estradiol, in a single dose, the bile being collected over a period of 72–96 hours; in the present study, alpha-estradiol was injected in doses of 10 mg. at 3-day intervals. The large variation in percentage recovery from the bile in the different periods is of interest in this connection (Table 1), the recovery during the first four periods being 49.6 per cent of the quantity injected, during the next three periods 10.9 per cent and during the last three periods 9.8 per cent. The discrepancy between the results of bioassay in the Philadelphia and Worcester laboratories is probably due to difference in procedure. In the former, the bile containing the mixture of estrogens was assayed against the administered solution of alpha-estradiol; in the latter, the three fractions were assayed against alpha-estradiol, estrone and estriol, respectively.

Whereas the recovery of estrogenic *activity* is relatively low in the present study, the recovery of estrogenic *material* is relatively high (39.3%) (Table

Received for publication January 30, 1945.

TABLE 1. (PHILADELPHIA LABORATORY) COLLECTION AND BIOASSAY OF BILE[1]

Period of collection	Volume of bile (cc.)	Bioassay (as microgram equivalents of alpha-estradiol)		Recovery (in percentage of each injection)
hours		Free	Conjugated	%
72	236	3,540	236	37.8
72	280	4,200	420	46.2
72	236	5,310	1,062	63.7
72	188	4,230	846	50.8
72	362	1,639	535	21.7
72	450	506	202	7.1
72	250	552	150	7.0
72	278	417	72	4.9
72	313	313	188	5.0
72	232	1,730	207	19.4
Total	2,825	22,437	3,918	26.4

[1] After intravenous injections of 10 mg. alpha-estradiol (in absolute alcohol) at the beginning of each collection period. Total amount injected 100 mg.

2). Inasmuch as the chemical studies were performed upon the pooled, 30-day specimen, no data are available regarding the relative recoveries in the individual 72-hour periods. Of particular interest is the fact that alpha-estradiol is not the principal constituent; a ketonic estrogen, presumably estrone, is preponderant. The presence of small amounts of a non-ketonic estrogen soluble in 0.3 M sodium carbonate, presumably estriol, is of interest

TABLE 2 (WORCESTER LABORATORY) FRACTIONATION OF THE FREE ESTROGENS OF THE BILE[1]

Estrogen fraction[1,2]	Estrogenic activity[3] (R.U.)	Calculated amount of estrogen[3] (mg.)	Actual weight of fraction (mg.)
"Estradiol"	99,000	7.9	50
"Estrone"	26,800	26.9	38
"Estriol"	4,500	4.5	34
Total	130,300	Total 39.3	
Total recovery	10.4%	39.3%	

[1] Obtained from the phenolic material of the bile by methods (Pearlman and Pincus 1943) previously employed in this laboratory; extraction of estrogen from the unhydrolyzed bile appears to have been complete since less than 4000 R.U. remained in the bile whereas a total of 130,300 R.U. are accounted for in the phenolic fractions.

[2] *Additional evidence* regarding the nature of the estrogen fractions (Table 2).

a. "Estradiol" fraction:—8 mg. of this fraction are digitonin-precipitable (theoretical 7.9 mg.).

b. "Estrone" fraction:—treatment with semicarbazide acetate resulted in over 98% inactivation.

c. "Estriol" fraction:—when this fraction was once more distributed between benzene and 0.3 M Na_2CO_3, 86.3% of the estrogenic activity was found in the carbonate phase and 13.7% in the benzene phase (cf. Mather, 1942).

[3] In the Worcester laboratory 0.08 microgram of alpha-estradiol, 1 of estrone, and 1 of estriol are equivalent respectively to one R.U.; the assays were kindly performed by M. R. J. Pearlman on spayed adult rats (Pincus and Pearlman, 1941) using the vaginal smear technique.

(also noted by Longwell and McKee, 1942). Further chemical study of the bile estrogens is warranted.

W. H. Pearlman[1]

The Physiological Laboratories
Clark University, Worcester, Massachusetts

K. E. Paschkis, A. E. Rakoff,
A. Cantarow, A. A. Walkling, L. E. Hansen

The Jefferson Medical College and Hospital
Philadelphia, Pennsylvania

REFERENCES

Cantarow, A., A. E. Rakoff, K. E. Paschkis, L. P. Hansen and A. A. Walkling: *Endocrinology* 31: 515. 1942.
Cantarow, A., K. E. Paschkis, A. E. Rakoff and L. P. Hansen: *Endocrinology* 33: 309. 1943.
Cantarow, A., A. E. Rakoff, K. E. Paschkis, L. P. Hansen and A. A. Walkling: *Proc. Soc. Exper. Biol. & Med.* 52: 256. 1943.
Gsell-Busse, M. A.: *Klin. Wchnschr.* 7: 1606. 1928; *Arch. f. d. ges. Physiol.* 219: 626. 1928; *Arch. f. exper. Path. u. Pharmakol.* 139: 328. 1929.
Longwell, B. B., and F. S. McKee: *J. Biol. Chem.* 142: 757. 1942.
Mather, A.: *J. Biol. Chem.* 144: 617. 1942.
Pearlman, W. H., and G. Pincus: *J. Biol. Chem.* 147: 379. 1943.
Pincus, G., and W. H. Pearlman: *Cancer Research* 1: 970. 1941.
Rakoff, A. E., A. Cantarow, K. E. Paschkis, L. P. Hansen and A. A. Walkling: *Endocrinology* 34: 370. 1944.

[1] Present address: Department of Biology, Princeton University; aided by a grant from G. D. Searle & Co.

THE EFFECT OF LIMITED WATER INTAKE ON BODY WEIGHT AND THYROID WEIGHT OF RATS TREATED WITH THIOURACIL[1]

Maintenance of animals at controlled environmental temperatures leads to a regulation of thyroid hormone production, presumably because alterations in the basal metabolism may compensate for changes in the rate of heat loss from the body (Dempsey and Astwood, 1943). Moreover, a considerable body of evidence indicates that a reduction in metabolism occurs during the emergency adjustments contingent upon hibernation, estivation or deprivation of water in animals, such as desert rats, which are capable of living on an essentially dry diet. Such reductions in metabolism, according to current endocrine theory, are brought about by a decrease in thyrotropic hormone production by the anterior pituitary gland.

The following experiments were undertaken to study the relation of reduced thyroid activity to the effects of a severely limited water intake, and the extent to which such restriction in water intake might alter the thyroid response to thiouracil.

MATERIALS AND METHODS

Male albino rats of the Wistar strain were used. The animals were approximately the same size and age, averaging 110 grams at the start of the

Received for publication March 1, 1945.
[1] This investigation was supported in part by a grant from the Committee on Endocrinology, National Research Council.

experiments. They were kept in separate cages at room temperature, fed a diet of finely ground Purina Fox Chow, and weighed daily. After asphyxiation with illuminating gas, the thyroid glands were removed, dissected free from fat and connective tissue, and weighed on a Roller-Smith torsion balance.

In the first experiment two groups of 12 rats each were given thiouracil, one group receiving water *ad libitum*, while the other group was given a lim-

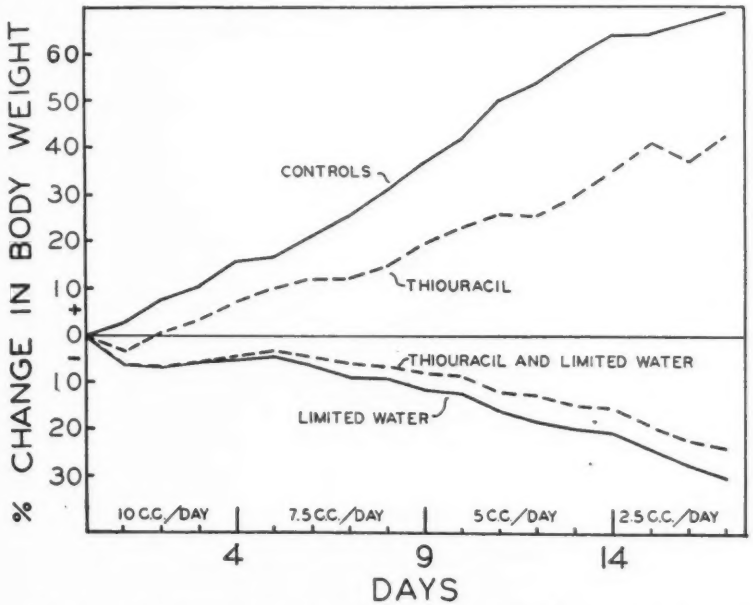

FIG. 1. Average percentage gain or loss in body weight for 36 rats in the second experiment. The two groups receiving limited water contained 12 rats each, the other 6 rats each, until 14 days when half of the animals were killed.

ited water ration. Three animals in each group were killed after 5, 8, 11, and 14 days, for the purpose of determining the rate of increase in weight of the thyroid gland.

In the second experiment 36 rats were divided into four groups. Two groups of 6 rats each were given water *ad libitum*, one group receiving thiouracil in addition. The other two groups of 12 rats each were given a limited water ration and one also received thiouracil. One-half of the rats in each group were killed after 14 days and the rest after 17 days. During this period the room temperature varied from 80° to 84° F., the average being 83° F.

All of the animals receiving thiouracil were given 0.1 per cent solution in their drinking water and 0.4 per cent dry powder mixed with their food. This provided a daily intake of approximately 64 mg. for rats in the unrestricted groups and a minimum of 20 mg. for those whose consumption of water

(and, consequently, of food) was limited. All of the animals given limited water rations received 10 cc. per day for the first four days, 7.5 cc. for the next five days, 5 cc. for the next five days, and 2.5 cc. for the last three days.

RESULTS

The percentage changes in body weight for the four groups of rats in the second experiment are shown in figure 1. A comparison of weight losses in the two limited water groups shows that the group which received thiouracil

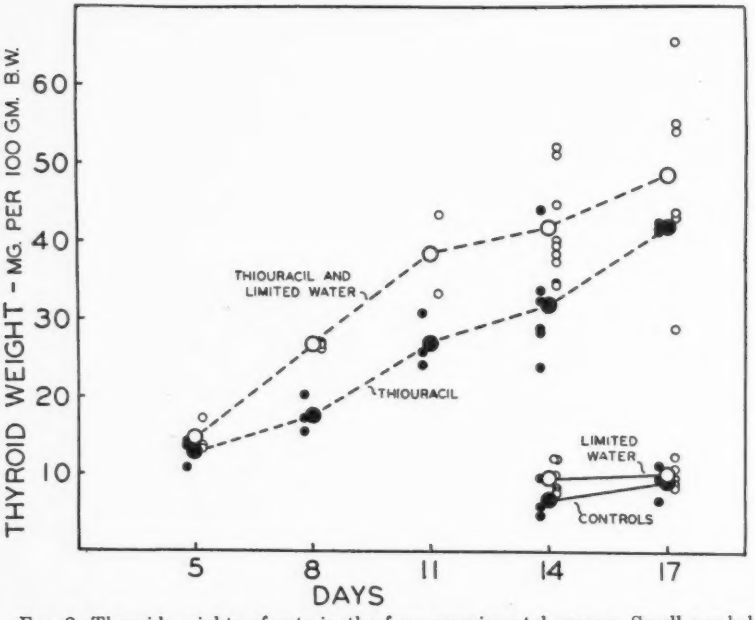

FIG. 2. Thyroid weights of rats in the four experimental groups. Small symbols indicate individual weights; large symbols, averages. (The averages of the absolute thyroid weights at 14 days were 40.2 mg. for the group on thiouracil and limited water, and 43.7 mg. for the group on thiouracil and unlimited water.)

had a small but definite advantage. This protection against weight loss became apparent at about the fifth day when the thyroxin reserves of the animals should have been exhausted. Differences between these two curves became statistically significant at 9 days and remained so until 14 days when half of the rats in each group were killed.

In the group of rats on limited water but receiving no thiouracil, one died on the seventh day, and four showed a red incrustation about the eyes and nose during the latter part of the experiment. The group which received thiouracil in addition to the limited water intake lived until the termination of the experiment and did not develop red incrustations.

As shown in figure 2, the relative thyroid weights for the two groups on restricted water intake were greater than those for the corresponding groups

given water *ad libitum*. It seems probable that this difference is apparent rather than real, due to the decreased body weight of the animals whose water intake was restricted. No satisfactory method of weighing this effect was found.

CONCLUSIONS

Rats receiving thiouracil were partially protected against the loss of weight resulting from a restricted water intake. The extent of protection and the time of its appearance suggested that it was due simply to the reduction in metabolism consequent to the elimination of the active thyroid hormone. The thyroid glands of rats given thiouracil and limited amounts of water were as large as, or larger than, those of rats receiving thiouracil and water *ad libitum;* hence the experiments failed to produce any evidence that the output of thyrotropic hormone was reduced in response to the restricted water intake.

CHARLES M. WALDO and EDWARD W. DEMPSEY
From the Department of Anatomy
Harvard Medical School and the Harvard
School of Dental Medicine, Boston, Mass.

REFERENCES

DEMPSEY, E. W., AND E. B. ASTWOOD: *Endocrinology* 32: 509. 1943.

ASSOCIATION NOTICE

ANNOUNCEMENT OF AWARDS

The Association for the Study of Internal Secretions has just announced that the award furnished by E. R. Squibb and Sons has been given to Dr. E. C. Kendall and that the award furnished by Ciba Pharmaceutical Products Inc. has been given to Dr. Jane Anne Russell (Mrs. A. E. Wilhelmi).

The Squibb Award to Dr. Kendall was based on his fundamental contributions to endocrinology, in particular the isolation of thyroxine from the thyroid gland and his recent observations on the fractionation and functions of active principles of the adrenal cortex. Dr. Kendall is professor of biochemistry in the Graduate School, Mayo Foundation, University of Minnesota. He received the degree of Bachelor of Science from Columbia University in 1908 and the degree of Doctor of Philosophy from the same institution in 1910. He then held various fellowships until he became affiliated with the Mayo Clinic in 1914. He was given the honorary degree of Doctor of Science by the University of Cincinnati in 1923, and was president of the Association for the Study of Internal Secretions in 1930.

The Ciba Award was made to Dr. Russell because of her fundamental observations on the role of the anterior pituitary, adrenal and thyroid glands in the absorption and utilization of sugars and starches. Dr. Russell is an instructor in physiological chemistry at Yale University School of Medicine. She received the degree of Bachelor of Arts from the University of California in 1932 and the degree of Doctor of Philosophy from the same institution in 1937. She has held various fellowships, the most important of which are the Porter Fellowship of the American Physiological Society, a National Research Council fellowship in medicine and the Sterling Fellowship in the Department of Physiological Chemistry at Yale University.

XUM